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Hyaluronate in Suction Blisters from Patients with Scleroderma and Various Skin Disorders

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With a specific radioassay hyaluronic acid (hyaluronate) concentrations have been determined in suction blister fluid from abdominal skin and serum. Healthy subjects, patients with acrosclerosis, CRST, mucinosis and urticaria had 0.8–5.6 μg/ml of hyaluronate in their suction blisters, which is about 100 times more than the serum level. Increased concentrations were noted in blister fluid from patients with active lesions of systemic and localized scleroderma as well as lichen sclerosus. Here the increase could be due to an increased production of hyaluronate in the dermis. High levels of hyaluronate were, however, also found in blister fluid from patients with other types of inflamed skin. An increased leakage into the blister of hyaluronate from the dermis, probably through the lymph vessels, therefore seems best to explain the high concentrations of hyaluronate. (Received February 15, 1986.)

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In patients with scleroderma an increase of glycosaminoglycans (GAG) have been reported in skin and urine (1–5). The GAG are in normal skin located mainly in the upper part of the dermis where hyaluronic acid, also called hyaluronate, and dermatansulphate are the main components. In scleroderma the ratio of hyaluronate to dermatansulphate was lower than in normal skin (6). Low levels of hyaluronate have been found in lesions of lichen sclerosus (7). Cultured fibroblasts from patients with active lesions of scleroderma have an increased biosynthesis and secretion mainly of hyaluronate (8–11). It could account for the high circulating level of hyaluronate found in scleroderma (12).

The aim of the present study was to find out if the level of hyaluronate in tissue fluid is influenced by different inflammatory conditions. We have therefore investigated the presence of hyaluronate in suction blister fluid from patients with connective tissue disorders and various skin diseases.

PATIENTS

Connective tissue disorders

The age, sex and diagnosis of the patients are shown in Table I. The patients were investigated before treatments. Patients 1 and 2 had an extensive lichen sclerosus of the trunk and proximal extremities progressing since 5–8 months with spontaneous blister formation on the abdomen.

Patients 3 and 4 had systemic scleroderma which within the last 3–6 months had spread to involve the trunk and upper arms. The test sites were slightly reddish-brown and infiltrated.

Patient 5: Since 4 years systemic scleroderma covering most of the body. He recently developed telangiectasia of the face and calcifications.

Patient 6: Moderate systemic scleroderma, telangiectasia and Raynaud's phenomenon for 10 years. Very slight involvement of abdominal test area.

Patients 7–10 had 2–8 typical plaques of localized morphea for 1–4 years. Tests were done on white plaques.

Patients 11–20 had sclerodactyly and Raynaud's phenomenon for 1–10 years. One had pulmonary involvement and some recent sclerodermic areas on the arms and buttocks.

Patients 22 and 23 calcinosis, Raynaud's phenomenon, sclerodactyly and telangiectasia (CRST syndrome). Patient 20 also had pulmonary involvement.

Patient 24 had systemic lupus erythematosus (SLE) and mucin-containing patches on the thorax from which a gelatinous material was sucked. Normal liver function.

Patient 25 had a typical reticular erythematous mucinosis (REM syndrome) on the back since several months.

Various disorders and healthy subjects

The patients and their diagnosis are listed in Table II. Patient 31 had an advanced tumorous type of mycosis fungoides where the blisters were raised on very red and irritated skin. The other patients with mycosis fungoides had more chronic lesions. They had some months previously been treated with PUVA bath. The other patients were investigated before any treatment was given with the exception of 2 patients with psoriasis who had been on oral etretinate (50 mg daily) for 2 months, respectively had 10 days of daily UV-B irradiation.

METHODS

Suction blister

The blisters were raised on the abdomen using the suction device described by Kiistala (13). In patients with diffuse scleroderma and morphea the tests were done on involved skin. In the others the skin appeared normal. A suction of 250–300 mm subpressure was used. The blisters appeared after 40–120 min in all patients except nos. 34 and 35 where only 15–20 min were needed. The clear yellowish blister fluid was taken out with a syringe, measured and immediately froozen and kept at −20°C until analyzed

Determination of hyaluronate

The hyaluronate concentration was determined by a slightly modified version of a previously designed specific radioassay (14) based on the specific affinity of cartilage proteins for hyaluronate. The method which is similar to a radioimmunoassay, has been modified for direct determinations of

Table I. *Hyaluronate in suction blister fluid from patients with connective tissue disorders*

The blisters were raised on abdominal skin in all except patient 23 where the back was examined

Pat. no.	Sex	Age	Diagnosis	Hyaluronate, μg/ml	
				Normal appearing skin	Diseased
1	F	67	Lichen sclerosus	–	54.9
2	F	74	Lichen sclerosus	–	34.7
3	F	68	Systemic scleroderma	–	14.8
4	F	64	Systemic scleroderma	–	10.2
5	M	18	Systemic scleroderma	–	8.4
6	F	56	Systemic scleroderma	–	1.6
7	M	71	Morphea	–	32.0
8	F	50	Morphea	–	15.0
9	F	69	Morphea	–	10.6
10	F	60	Morphea	–	8.8
11–21	F, M	24–83	Acrosclerosis	0.9–5.6	–
22–23	F	61–80	CRST	0.7–2.4	–
24	M	40	Mucinosis + LED	–	4.2
25	F	39	REM syndrome	0.8	6.7

hyaluronate in serum (15). In the present study the blister solution was diluted twenty times with 0.025 M phosphate buffer pH 7.0, and the following solutions were mixed in 1.5 ml centrifuge tubes (Micro tests tubes 3810, Eppendorf, Gerätenbau, Hamburg, FRG) for standard curves: 1) 100 µl diluted blister solution or 100 µl sample buffer. 2) 400 µl physiological saline containing 60 g/l of bovine serum albumin (Fraction V; Sigma, St. Louis, MO, USA pH 7.5) or this solution containing 2 ng–400 ng hyaluronate (Healon®, Pharmacia, Uppsala, Sweden). Otherwise the blister fluid and the serum samples were analysed according to the same procedure as outlined by Engström-Laurent et al. (15).

RESULTS

The level of hyaluronate in blister fluid of patients with connective tissue disorders appears in Table I. The highest levels were noted in blister fluid from patients with active lesions of both systemic and localised scleroderma as well as two patients with lichen sclerosus. In one patient (no. 6) with systemic scleroderma for many years and minor involvement of the tested site the level of hyaluronate was low. Of those with sclerodactyly and Raynaud's syndrome the highest level (5.6 µg/ml) was found in a patient who also had recent pulmonary involvement.

Increased levels of hyaluronate were obtained in blister fluid from lesional skin of some patients with mycosis fungoides, eczema, atopic dermatitis and treated psoriasis (Table II). In some of them also the normal appearing skin showed elevated hyaluronate levels.

The serum levels of hyaluronate were investigated in 14 of the patients. In 4 patients with acrosclerosis and CRST the serum levels were 0.05–0.12 µg/ml. An increase was seen in patients 3 and 4 with diffuse scleroderma (0.35 and 0.58 µg/ml) and in patient 50 with cirrhosis (0.52 µg/ml). Healthy subjects and patients 24–25 had 0.01–0.04 µg/ml.

Table II. *Hyaluronate in suction blister fluid from abdominal skin of patients with various disorders and healthy subjects*

Pat. no.	Sex	Age	Diagnosis	Hyaluronate, µg/ml	
				Normal appearing skin	Lesion
26	M	73	Prurigo nodularis	3.2	26.2
27	M	21	Atopic dermatitis	14.4	13.6
28	F	42	Atopic dermatitis	8.4	25.6
29	F	63	Eczema (generalized)	–	10.6
30	M	62	Eczema (generalized)	–	42.0
31	M	78	Mycosis fungoides	11.3	34.7
32	F	74	Mycosis fungoides	3.9	7.4
33	M	63	Mycosis fungoides	2.8	3.1
34	F	64	Mycosis fungoides	4.1	–
35	M	57	Mycosis fungoides	5.3	–
36	M	62	Psoriasis-plaque Etretinate	26.7	26.2
37	M	19	Psoriasis UV-B	12.6	19.3
38	F	35	Urticaria pigmentosa	–	2.0
39	F	62	Urticaria chronic	–	1.0
40	F	42	Adipositas dolorosa Dercum	5.0	–
41	M	64	Alcoholic cirrhosis	3.6	–
42	F	39	Healthy subjects	3.6	–
43	M	57	Healthy subjects	3.2	–
44	M	74	Healthy subjects	1.0	–

DISCUSSION

Increased levels of circulating hyaluronate have been observed in patients with localized or systemic scleroderma and in patients with rheumatoid arthritis (12, 16). The elevated serum levels were considered to be due to an increased synthesis of hyaluronate in the inflamed tissues. It has also been shown that patients with cirrhosis of the liver have elevated serum levels of hyaluronate due to an impaired elimination of the polysaccharide by the endothelial cells of the liver (17). Increased serum levels of hyaluronate have also been reported in patients with psoriasis (18). The highest values were found in patients with widespread active disease and/or active arthritis.

The concentration of hyaluronate in suction blister fluid is about 100 times higher than in serum. It is therefore not likely that a 2–10 times increase in serum will at all influence the hyaluronate levels in the blister fluid. This assumption is strengthened by the finding that the patient with liver cirrhosis and a high serum hyaluronate value had normal concentrations in the blister fluid.

In blister fluid from patients with lichen sclerosus and scleroderma the hyaluronate was mainly increased in new active lesions. It was low in normal appearing skin of patients with acrosclerosis or CRST. The increase of hyaluronate concentration in blister fluid was not specific for patients with scleroderma. High levels of hyaluronate were also found in various inflammatory skin disorders such as psoriasis (19), mycosis fungoides, atopic dermatitis and eczema. In some of these patients the increase was also noted in normal appearing skin. Here the normal appearing skin probably was changed by the disease, since the patients had not received any treatment. In a group of non-treated patients with psoriasis no such increase was observed in normal appearing skin whereas the lesions had elevated hyaluronate levels in the blister fluid (19). That various irritants can increase the leakage of hyaluronate from non-lesional skin is probably the case in patients 36–37 treated with etretinate respectively UV-B irradiation. When hyaluronate was determined in skin biopsies as uronic acid with another method the concentration was the same in psoriatic and healthy skin (20). The explanation to the apparent difference could be that it is the leakage of hyaluronate into the blister fluid and not the absolute concentration in the skin which is increased. In patients with connective tissue disorders an increased production of hyaluronate of fibroblasts from active lesions (11) could have contributed to its high level in our suction blisters although an increased leakage could even here be the main reason.

The source of hyaluronate in the suction blister is most probably the dermis since the plasma and serum levels are 100 times lower and only trace amounts have been detected in the epidermis (6). An increased leakage through inflamed skin seems, as discussed above, to be the explanation for the increase in some of the disorders studied. This could take place through the lymph vessels which are dilated in inflammatory conditions such as psoriasis and various forms of eczema (21). In such disorders they can also be dilated in the normal appearing skin. Such a possibility is favoured by the recent finding of high levels of hyaluronate in the lymph (22, 23). The time for producing suction blisters could here also be of importance but we found no major difference between lesional and normal appearing skin.

REFERENCES

1. Uitto J, Ohlenschlager K, Lorenzen IB. Solubility of skin collagen in normal human subjects and in patients with generalized scleroderma. Clin Chim Acta 1971; 31: 13–18.
2. Fleischmajer R, Perlish J. Glycosaminoglycans in scleroderma and scleredema. J Invest Dermatol 1972; 58: 129–132.
3. Hardy KH, Rosevear JW, Sams WM, Winkelmann RK. Scleroderma and urinary excretion of acidic glycosaminoglycans. Mayo Clin Proc 1971; 46: 119–127.

4. Blumenkrantz N, Asboe-Hansen G. Variation of urinary acid glycosaminoglycan and collagen metabolite excretion with disease activity in generalized scleroderma. Acta Derm Venereol (Stockh) 1980; 60: 39–43.
5. Murata K, Takeda M. Compositional changes of urinary acidic glycosaminoglycans in progressive systemic sclerosis. Clin Chim Acta 1980; 108: 49–59.
6. Tajima S, Nishikawa T, Hatano H, Nagai Y. Distribution of glycosaminoglycans in dermal connective tissue from scleroderma patients. J Dermatol 1982; 9: 405–408.
7. Yoshima H, Shinkai H, Sano S, Homma, M. Acidic glycosaminoglycans in skin and urine of a patient with wide-spread lichen sclerosus et atrophicus. Arch Dermatol Res 1970; 263: 59–66.
8. Buckingham RB, Prince RK, Rodnan GP. Progressive systemic sclerosis (PSS, scleroderma) dermal fibroblasts synthesize increased amounts of glycosaminoglycan. J Lab Clin Med 1983; 101: 659–669.
9. Cabral A, Castor CW. Connective tissue activation. XXVII. The behavior of skin fibroblasts from patients with scleroderma. Arthritis Rheum 1983; 26: 1362–1369.
10. Bashey RI, Millan A, Jimenez SA. Increased biosynthesis of glycosaminoglucans by scleroderma fibroblasts in culture. Arthritis Rheum 1964; 27: 1040–1050.
11. Vuorio TK, Kähäri V-M, Lehtonen A, Vuorio El. Fibroblast activation in scleroderma. Scand J Rheum 1984; 13: 229–237.
12. Engström-Laurent A, Feltelius N, Hällgren R, Wasteson Å. Elevated serum hyaluronate in scleroderma. An effect of growth factor-induced activation of connective tissue cells? Ann Rheum Dis 1985; 44: 614–620.
13. Kiistala U. Suction blister device for separation of viable epidermis from dermis. J Invest Dermatol 1968; 50: 129–137.
14. Tengblad A. Quantitative analysis of hyaluronate in nanogram amounts. Biochem J 1980; 185: 101–105.
15. Engström-Laurent A, Laurent UBG, Lilja K, Laurent TC. Concentration of sodium hyaluronate in serum. Scand J Clin Lab Invest 1985; 45: 497–504.
16. Engström-Laurent A, Hällgren R. Circulating hyaluronate in rheumatoid arthritis. The relationship to inflammatory activity and the effect of corticosteroid therapy. Ann Rheum Dis 1985; 44: 83–88.
17. Engström-Laurent A, Lööf L, Nyberg A, Schröder T. Increased serum levels of hyaluronate in liver disease. Hepatology 1985; 5: 638–642.
18. Lundin Å, Engström-Laurent A, Hällgren R, Michaëlsson G. Circulating hyaluronate in psoriasis. Br J Dermatol 1985; 112: 663–671.
19. Lundin Å, Engström-Laurent A, Michaëlsson G, Tengblad A. High levels of hyaluronate in suctionblister fluid from active psoriasis lesions. Br J Dermatol (in press).
20. Poulsen JH, Cramers MK. Biochemical analysis of dermal glycosaminoglycans in psoriasis. Br J Dermatol 1982; 107: 569–570.
21. Braverman IA. The role of blood vessels and lymphatics in cutaneous inflammatory processes: an overview. Br J Dermatol 1983; 109: Suppl 25: 89–98.
22. Laurent UBG, Laurent TC. On the origin of hyaluronate in blood. Biochem Internat 1981; 2: 195–199.
23. Tengblad A, Laurent UGB, Lilja K, Cahill RNP, Engström-Laurent A, Fraser JRR, Hansson, HE, Laurent TC. Concentration and molecular weight of hyaluronate in lymph and blood. Biochem J. Accepted for publication.