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OSTEOARTHRITIS and CARTILAGE

Growth factors, insulin-like growth factor-1 and growth hormone, in synovial fluid and serum of patients with rheumatic disorders

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Summary

Objective: Synovial fluid (SF) plays an important role in joint function. We evaluated the growth factors, insulin-like growth factor-1 (IGF-1) and growth hormone (GH) in SF and serum from patients with osteoarthritis (OA), rheumatoid arthritis (RA), gout, pseudogout and diffuse idiopathic skeletal hyperostosis (DISH).

Design: Standard radioimmunoassay techniques were used to measure concurrent levels of IGF-1 and GH. SF samples and serum samples were obtained concomitantly from 27 patients with OA, 22 patients with RA, nine men with gout, 14 patients with pseudogout and eight men with DISH.

Results: In the case of IGF-1, a comparison of serum and SF levels shows that SF levels of IGF-1 are lower than serum levels in all groups. Men and women gave similar values. In contrast, in the case of GH, all groups, except males with RA, had higher GH values in SF when compared with serum values. Individual patients with other forms of arthritis demonstrated similar relationships.

Conclusion: The finding that IGF-1 is present in levels about one-half as great in SF as compared with serum suggests that IGF-1 may be produced in lesser amounts or is utilized by the patient in customary joint function. The finding that GH is present in SF at values twice as high, or more, of serum levels in inflammatory arthritides suggests that GH may play a role in the pathophysiology of arthritic disorders.

Keywords: Synovial fluid, Growth hormone, Insulin-like growth factor-1.

Introduction

Synovial fluid (SF) plays an important role in joint function, facilitating movement, and providing joint nutrients [1]. It contains soluble constituents such as proteins and electrolytes derived from blood, and macromolecules secreted by joint tissues such as hyaluronic acid and lubricating glycoproteins. SF from patients with rheumatoid arthritis (RA) and from patients with osteoarthritis (OA) contains acute phase reactants, transferrin, ceruloplasmin, albumin, antitrypsin, acid glycoprotein, immunglobulins G, A, M and beta-endorphin [2]. SF levels of substance P, a proinflammogen, exceed plasma levels in RA, OA, and post-traumatic arthritis [3].

Recently, we reported perturbations of insulin-like growth factor (IGF-1) and growth hormone (GH) in the serum of patients with OA [4] and diffuse idiopathic skeletal hyperostosis (DISH) [5]. In preliminary studies of SF, we found levels of glucose in SF approximated serum levels; IGF-1 levels were lower and GH levels exceeded those in serum [6]. In the present study, we evaluated concomitant levels of the growth factors, IGF-1 and GH, in the SF and serum of patients with rheumatic disorders. We sought further evidence in support of the hypothesis that these growth factors play a role in the pathophysiology of the rheumatic disorders.

Patients

The patients were volunteers recruited from the Arthritis Clinics of University Hospitals of Cleveland and Wade Park Veterans Administration Hospital in Cleveland, U.S.A. No patient in this group was included in any previous publication on biochemical changes in SF in rheumatic disorders. The study was approved by the Institutional Review Boards of both institutions. Diagnostic guidelines were based on the criteria in 'Primer on the Rheumatic Diseases' [7] for RA, systemic lupus erythematosus, (SLE), and gout. OA patients met the following criteria: age over 45 years, clinical symptoms of pain and stiffness, joint swelling and characteristic

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radiologic changes of joint space narrowing and osteophytes in symptomatic joints. Patients with DISH were older than 45 years, with clinical symptoms of pain and stiffness in the spine and characteristic radiological changes of exuberant osteophytes in the spine with anterolateral ossification and calcification of ligamentous spinal processes. All DISH patients studied had knee effusions. All patients had active disease manifested by knee effusions. Arthrocentesis was performed for diagnosis or for treatment. Blood was drawn by venipuncture shortly after joint fluid was obtained. Treatment programs included nonsteroidal anti-flammatory drugs (NSAIDs) and analgesic agents, corticosteroids, inhibitors of uric acid synthesis, immunosuppressants, and colchicine. Of 24 patients with RA, only seven received corticosteroids regularly in their treatment, four males and three females. Comparison was made with normal controls contained in studies previously reported from this laboratory [4, 5].

Material and methods

Blood was drawn by venipuncture for assays of IGF-1 and GH in the serum. This was done usually a few minutes after SF was withdrawn from the knee. SF obtained from joints other than the knee was not included in the study.

Blood and SF samples were treated in similar fashion. Blood was clotted at room temperature, then centrifuged and assayed. Serum glucose levels were determined. Blood samples were used if the glucose level was normal, 65–130 mg/dl. Samples were obtained usually between 9.30 a.m. and 12.30 p.m.

Standard radioimmunoassays (RIA) (INCSTAR, Stillwater, MN, U.S.A.) were adapted for GH and IGF-1. The manufacturer provided quality control specimens for each peptide with each kit for each run; assays were validated by our laboratory.

GH

The GH assay is a disequilibrium RIA using addition of sample and guinea-pig anti-human serum followed by incubation. [^{125}I]-GH is then added, followed by a second incubation. Preprecipitated carrier, second antibody and polyethylene glycol (PEG) are added in a single step (INCSTAR manual for GH 07130). Our GH assay was adapted for low values by using a log-logit calculation mode on a programmable Beckman 5500 gamma counter attached to a computer which prints the lowest detectable dose.

Quality control evaluation for GH RIA gave a coefficient of variance as follows: for low values <2 ng/ml, 10 interkit assays 10.5%, 10 intrakit assays 6.8%. Cross reactivity of the human GH antibody was less than 0.8% for the following human peptides: insulin, beta-endorphin, prolactin, ACTH, placental lactogen, chorionic gonadotrophin, thyroid stimulating hormone, luteinizing hormone, follicle stimulating hormone. The minimal detectable amount was 0.4 ng/ml.

IGF-1

The IGF-1 assay is a double antibody disequilibrium RIA which includes an octadecasilyl-silica column extraction of serum and SF. After extraction, the RIA is performed by adding sample and rabbit anti-IGF-1 serum, followed by an incubation of 2 h. Then [^{125}I] tracer is added and incubated for 20 h. Then the precipitating complex and PEG are added in one step and incubated for 2 h. (INCSTAR manual for IGF-1, catalog No. 53065.) Quality control studies gave the following results: intrakit CV, 10 assays, 8%; interkit CV, 10 assays 5.6%. This IGF-1 antibody exhibited less than 0.01% crossreactivity with IGF-II. Minimal detectable amount was <2.0 nM/l.

GLUCOSE

Glucose was measured by the hexokinase method. Other hexoses such as fructose and mannose were detected but they were present only in trace amounts. Quality control evaluation gave the following results: 10 assays, interkit coefficient of variation (CV) 8%, intrakit 10 assays CV 8%.

For evaluation of data we used a paired *t*-test comparing the SF with serum levels of each hormone in each patient.

Results

The serum and SF levels of IGF-1 and GH in groups of patients with RA, OA, gout, pseudogout and DISH are presented in Table I. A comparison of serum and SF levels of IGF-1 shows that the serum levels are higher than SF levels in all groups studied. GH levels, on the other hand, were distinctly different from the IGF-1 findings. All patient groups demonstrated GH levels in the SF to be significantly higher than the levels in the serum, except for male patients with RA. SF glucose levels were similar to serum values (data not shown).

Discussion

Interrelations of hormones and rheumatic disorders have been widely studied encompassing natural human rheumatic diseases and arthritis induced in experimental animals under carefully regulated laboratory conditions. Although the role of growth factors, GH and IGF-1, is not completely understood, it is obvious that they have more than the function of being generalized growth promoters. GH plays an essential role in the pathogenesis of acromegalic arthritis. Only when treatment reduces serum levels of GH to normal levels is there a reduction of joint pain and stiffness in the patient [8–10]. Several decades ago, Reinhart and Li [11] demonstrated hypophyseal GH induced an arthritis in rats that had features of both OA and RA. Observations in rats and tissue culture experiments indicate that excessive GH inhibits cartilage metabolism [12, 13]. Adjuvant arthritis cannot develop in rats unless there is a source of GH or prolactin [14], a compound with growth promotion similar to GH [15]. Excess GH occurs in women with OA [16, 17]. Patients with radiologic changes of OA, but without clinical symptoms, have normal levels of growth factors [18].

A recent report suggests that hepatocyte growth factor (HGF) in SF of patients with RA is produced by SF cells, and is related to disease activity in RA [19]. This growth factor in SF from patients with RA was high enough to stimulate DNA synthesis in human hepatocytes. It is a potent mitogen for mature hepatocytes, and plays an essential role in the function of many cell types, possibly being involved in synovial inflammation as well.

Another relation of rheumatic disorders and growth factors, especially GH, comes from the studies of Vanhagen and co-workers [20] on somatostatin receptor, a potent inhibitor of GH release. Arthritic clinical activity in RA and OA correlates with somatostatin receptor in swollen and painful joints. When somatostatin was injected into painful RA knees, symptoms improved.

Many effects of GH are considered to be mediated through IGF-1, produced by the liver when stimulated by GH. Although IGF-1 is produced by many tissues [21], the liver is the main source [22]. IGF-1 and GH levels are influenced by counter-balancing mechanisms. When GH levels increase, thereby stimulating IGF-1 production, the increased IGF-1 levels, in turn, tend to suppress further synthesis of GH. IGF-1 thus exerts an anti-inflammatory effect, as well as stimulating growth of tissue (cartilage). IGF-1 couples with binding protein, and may be excessively bound in active stages of arthritis disorders.

Dore and coworkers [23] reported a single class

Table I

Insulin-like growth factor-1 (IGF-1) and growth hormone (GH) in synovial fluid (SF) and serum (S) of patients with rheumatic disorders (mean ± S.D.)

Group	Gender	Source	*N*	IGF-1 (nM/l)	S/SF	*P**	GH (ng/ml)	S/SF	*P**
Osteoarthritis (27)	Male	S	12	14.9 ± 4	1.8	0.001	1.2 ± 0.5	0.6	0.002
		SF		9.4 ± 4			2.4 ± 1.0		
	Female	S	15	10.9 ± 2	1.7	0.001	1.5 ± 0.1	0.6	0.001
		SF		6.4 ± 2			2.1 ± 0.8		
Rheumatoid arthritis (24)	Male	S	13	20.6 ± 7	1.6	0.001	1.8 ± 2	1.1	NS
		SF		12.7 ± 4			1.6 ± 0.9		
	Female	S	11	19.4 ± 5	1.8	0.001	1.1 ± 0.4	0.3	0.015
		SF		10.7 ± 4			3.3 ± 3		
Gout (9)	Male	S	9	15.7 ± 4	1.5	0.005	0.7 ± 0.3	0.4	0.013
		SF		10.7 ± 6			1.8 ± 1.6		
Pseudogout (14)	Male	S	7	13.7 ± 3	2.4	0.001	0.97 ± 0.2	0.6	0.019
		SF		5.6 ± 0.4			1.5 ± 0.3		
	Female	S	7	18.2 ± 3	1.8	0.006	0.99 ± 0.5	0.4	0.007
		SF		11.0 ± 4			2.6 ± 0.3		
DISH (8)	Male	S	8	22.8 ± 8	2.2	0.003	0.91 ± 0.3	0.4	0.001
		SF		9.4 ± 2			2.5 ± 0.6		
Normal	Male	S (W)†	22	22.0 ± 7			0.9 ± 0.3		
	Female	S (W)†	22	16.5 ± 3			1.1 ± 0.2		
		S (B)†	10	22.8 ± 5			0.8 ± 0.5		

*P = paired t-test, SF vs S for each patient.
†W = White.
†B = Black.

of binding sites in human OA chondrocytes and normal chondrocytes. OA chondrocytes had a lower affinity and a higher density compared with normal cells. Immunohistochemical studies with monoclonal antibody against Type 1 IGF receptor showed increased staining compared with normal tissue. However, OA chondrocytes were unresponsive to IGF stimulation. Increased IGF binding protein on the cell surface may also diminish bioavailability and reduce anabolic action. Coates and coworkers have reported decreased levels of IGF-1 (somatomedins) in diverse arthritides [24].

GH is associated with joint symptoms such as pain and swelling; accordingly high levels of GH in a joint would appear to be undesirable. Excess growth-factor production by synovial fibroblasts may play an important role in inflammatory states, as well as influencing the formation of rheumatoid pannus [25, 26]. Treatment programs may benefit the patient by altering these hormonal imbalances. A common therapeutic measure in treating patients with RA is use of corticosteroids, agents that have been shown to counteract actions of GH, especially in regard to cartilage metabolism [27]. The variation in serum:SF ratios which we observed between men and women, although unexplained at this time, may lie in differences in basic physiology between men and women or gender variations in response to RA. Although the relationship of our observations to disease pathogenesis and pathophysiology require further study as to clinical implications, such studies related to interplays between growth factors, inflammation and joint tissue responses will hopefully provide clues not only to mechanisms but to new therapeutic approaches. In this regard, further assessment of the symptoms of absolute amounts of specific hormones in SF, irrespective of serum: SF ratios would be of interest.

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References

1. Swann DA. Macromolecules of synovial fluid. In: Sokoloff L, Ed., Vol. 1. The Joints and Synovial Fluid. New York: Academic Press 1978:407–35.
2. Denko CW, Aponte J, Gabriel P, Petricevic M. Beta-endorphin, immunological and biochemical changes in synovial fluid in rheumatic disorders Clin Rheumatology 1986;5:25–32.
3. Marshall KW, Chin B, Inman RD. Substance P and arthritis: analysis of plasma and synovial fluid levels. Arthritis Rheum 1990;33:87–90.
4. Denko CW, Boja B, Moskowitz RW. Growth promoting peptides in osteoarthritis: insulin, insulin-like growth factor-1, growth hormone J Rheumatol 1990; 17: 1217–21.
5. Denko CW, Boja B, Moskowitz RW. Growth promoting peptides in osteoarthritis and diffuse idiopathic skeletal hyperostosis—insulin, insulin-like growth factor-1, growth hormone. J Rheumatol 1994;21:1725–30.
6. Denko CW, Boja B, Moskowitz RW. Synovial fluid levels of insulin-like growth factor-1 and growth hormone in osteoarthritis and rheumatoid arthritis. [Abstract] Arthritis Rheum 1991;34:r28.
7. Schumacher RH Jr, Klippel JH, Robinson DR, Eds. Primer of Rheumatic Diseases 9th ed, Atlanta: Arthritis Foundation 1988.
8. Lacks S, Jacobs RP. Acromegalic arthropathy: a reversible rheumatic disease. J Rheumatol 1986;13:534–6.
9. Layton MW, Fudman EJ, Barkan A. Braunstein EM, Fox IH. Acromegalic arthropathy. Arthritis Rheum 1988;31:1022–7.
10. Podgorski M, Robison B, Weissberger A, Stiel J, Wang S, Brooks PM. Articular manifestation of acromegaly. Aust NZ J Med 1988;18:28–35.
11. Reinhardt W, Li CH. Experimental production of arthritis in rats by hypophyseal growth hormone. Science 1953;107:295–7.
12. Denko CW, Bergenstal DM. The effect of hypophysectomy and growth hormone on ^{35}S fixation in cartilage. Endocrinology 1955;57:76–86.
13. Smith TWD, Duckworth T, Bergenholtz A, Lempberg RK. Role of growth hormone in glycosaminoglycan synthesis by articular cartilage. Nature 1975;253:269–71.
14. Berczi I, Nagy E, Ash S, Kovacs K. The influence of pituitary hormones on adjuvant arthritis. Arthritis Rheum 1984;27:682–93.
15. Denko CW. The effect of prolactin on S^{35} fixation in the costal cartilage of the hypophysectomized rat. Endocrinology 1959;65:147–51.
16. Franchimont P, Denis F. Determination du taux de somatotrophines dans cas d'arthrose apparaissant lors de menopause. J Belge Rheumatol Med Phys 1968;23:59–64.
17. Dequeker J, Burssens A, Bouillon R. Dynamics of growth hormone secretion in patients with osteoporosis and in patients with osteoarthrosis. Hormone Res 1982;16:353–6.
18. Denko CW, Boja B. Growth factors in asymptomatic osteoarthritis—insulin, insulin-like growth factor-1, growth hormone. Inflammapharmacology 1993;2:71–6.
19. Yukioka K, Inaba M, Furimitsu Y, Yukioka M, Nishind T, Goto H, Nishizawa Y, Morii H. Levels of hepatocyte growth factor in synovial fluid and serum of patients with rheumatoid arthritis and release of hepatocyte growth factor by rheumatoid synovial fluid cells. J. Rheumatol 1994;21:2184–9.
20. Vanhagen PM, Markusse HM, Lamberts SWJ, Kwekkeboom DJ, Reubi JE, Krenning EP. Somatostatin receptor imaging the presence of somatostatin receptors in rheumatoid arthritis. Arthritis Rheum 1994;37:1521–27.
21. Leroith D. Moderator, Insulin-like growth factors in

health and disease. Ann Intern Med 1992;116:854–62.
22. Johnson TR, Blossy BK, Denko CW, Ilan J. Expression of insulin-like growth factor-1 in cultured rat hepatocytes: effects of insulin and growth hormone. Molecular Endocrinol 1989;3: 580–7.
23. Dore S, Pelletier JP, DiBattista JA, Tardif G, Brazeau P, Martel-Pelletier J. Human osteoarthritic chondrocytes possess an increased number of insulin-like growth factor-1 binding sites but are unresponsive to its stimulation—possible role of IGF-1 binding proteins. Arthritis Rheum 1994;37:253–63.
24. Coates CL, Burwell RG, Lloyd-Jones K, Swannell AJ, Walker G, Selby C. Somatomedin activity in synovial fluid from patients with joint diseases. Ann Rheum Dis 1978;37:303–14.
25. Anastassiades TP, Ley J, Wood A, Irwin D. The growth kinetics of synovial fibroblasts from inflammatory and non-inflammatory arthropathies. Arthritis Rheum 1978;21:461–7.
26. Espinoza LR, Espinoza CG, Chellar ML, Scopelitis E, Silveira LH, Grotendorst CG. Fibroblast function in psoriatic arthritis II. Derived growth factor and increased production of growth factor and cytokines. J. Rheumatol 1994;21:1507–11.
27. Denko CW, Bergenstal DM. Effects of growth hormone and corticosteroids on ^{35}S fixation in cartilage. Endocrinology 1961;69:769–77.