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METABOLIC DISTURBANCES AND SYNOVIAL JOINT RESPONSES IN OSTEOARTHRITIS

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1. ABSTRACT

Previously held views that the pathogenesis of idiopathic osteoarthritis (OA) originated in the synovial joint and was not influenced by systemic metabolic disturbances in the patient is inconsistent with recent data demonstrate skewing of the growth hormone/insulin-like growth factor-1 axis in the symptomatic OA patient. In light of this novel information, the role of growth hormone and insulin-like growth factor-1 in the pathogenesis and progression of OA requires further definition. In male patients with OA, the red blood cell sequesters more growth hormone than an aged-matched control group. Thus, this growth hormone "depot" may provide a mechanism for removal of "toxic" levels of growth hormone from the circulation. Storage of "excess" growth hormone in red cells may reduce the inflammatory or otherwise undesirable "toxic" actions of GH. In some patients, serum growth hormones levels may exceed three-times the average value considered normal. These "episodic" variations in growth hormone levels may play a significant role in the elevated levels of serum growth hormone seen in the OA patient. The connection between elevated growth hormone and decreased insulin-like growth factor-1 levels and the defined cartilage anabolic and catabolic pathways defined in *in vitro* assays of articular cartilage derived from the OA patients remain to be more precisely defined. However, the dampened insulin-like growth factor-1 response in OA coupled with elevated cartilage extracellular matrix degradation (mediated by metalloproteinases) and depressed compensatory biosynthesis (induced and perpetuated by the presence of cytokines such as interleukin-1 and tumor necrosis factor-alpha) may, in fact, act synergistically to suppress normal cartilage repair mechanisms thus resulting in progressive destructive lesions of the cartilage and bone.

2. INTRODUCTION

Our hypothesis, that the most common rheumatic disorder, idiopathic osteoarthritis (OA) contains features that could classify it as a metabolic disturbance requires that deviations within the individual patient with OA be explored. Since the definition of "normal" may not be agreed upon universally in characterizing the patient without the diagnosis of OA, so it is with the view of what constitutes "abnormal" in the patient with OA. In this respect, we will use a definition of OA formulated on the basis of the criteria established by the American College of Rheumatology (ACR) covering OA of the hip and knee (1). Involvement of the spine is based on similar criteria. Since OA is a generalized disorder, each specific joint involved is part of the whole constellation of joint abnormalities with minor modifications characteristic of each joint. These minor modifications are probably a result of the interaction of systemic and local factors with individual synovial joint components. The common complaint of pain in the afflicted joint will depend on the sensitivity and perspicacity of each patient. In addition to pain in specific joints, patients must also demonstrate evidence of morphological cartilage and bone changes by physical or radiographic examination.

3. CLINICAL FINDINGS RESULTING IN THE DIAGNOSIS OF OA

There are essentially no distinguishing differences in the physiochemical reactions of chondrocytes and in the properties of articular cartilage in various rheumatic disorders for these criteria to be useful in the classification of these patients. Therefore, it is helpful to turn to the clinical features of the disease to aid in its classification. Is there sufficient sameness in OA when individual cases of OA are compared to other rheumatic disorders or connective tissue diseases to justify the viewpoint that OA is fundamentally a metabolic disorder? The ACR criteria for the diagnosis of OA requires that a combination of clinical features be present (1). Specific criteria for hip and knee OA include a combination of clinical history, physical examination, laboratory and

radiographic studies. In all forms of OA, arthritis must be defined by the presence of joint pain, aching or stiffness. In hip and knee OA, bony tenderness and enlargement must be present with the additional finding of crepitus contributing to the diagnosis of OA in the knee. X-rays of the hip must show evidence of joint space narrowing and/or the presence of osteophytes.

4. LABORATORY FINDINGS IN OA AND IN INFLAMMATORY DISEASES

Laboratory tests should reveal normal sedimentation rates, no rheumatoid factor and normal synovial fluid viscosity and lubrication capacity. In studies from this laboratory, in patients 45 years or older, changes in acute phase reactant levels produced in inflammatory disorders such as rheumatoid arthritis, gout, pseudogout, and systemic lupus erythematosus (SLE) were similar to those measured in patients with clinically diagnosed OA (2). Thus, the inflammatory component in these common rheumatic disorders is similar and is often medically managed by the use of corticosteroids, and/or non-steroidal anti-inflammatory drugs (NSAIDs). In specific cases such as in the use of colchicine for the treatment of gout, agents with more specific targets of action are more medically effective than agents with broad basis of action such as NSAIDs. We and others have postulated that changes in acute phase reactants are protective (3-7). Transferrin and albumin are carrier proteins whose serum levels are reduced in patients with OA thereby effectively reducing the overall impact of the inflammatory component on the disease process. Other acute phase proteins whose anti-inflammatory activities depend on increasing serum levels include ceruloplasmin which also serves as a carrier protein for copper (4), acid glycoprotein (5), and alpha$_1$-antitrypsin (6). Beta-endorphin, a 31-amino acid neuro-transmitter protein is the biochemical mechanism that integrates pain and euphoria (7). Beta-endorphin results from the conversion of a larger glycoprotein, proopiomelanocortin which is also the precursor for other hormones such as adrenocorticotropin. Endorphins were shown to stimulate cartilage metabolism *in vitro* (8). Serum endorphin levels in patients with OA were low compared to normal individuals as was also the case with other inflammatory disorders such as gout, pseudogout, rheumatoid arthritis, SLE, psoriatic arthritis and ankylosing spondylitis (9).

These patterns of systemic biochemical changes are similar to those occurring with acute phase reactants. The acute phase reactants which are synthesized in the liver are rapidly induced in the presence of inflammatory stimuli and appear to parallel states of chronic inflammation (10).

4.1. Growth hormone and insulin-like growth factor-1

The role of growth factors and other potential mediators of growth factor activity in the pathogenesis and progression of OA has been considered (11) but the exact mechanism of action remains to be established. We and others previously demonstrated that elevated serum growth hormone (GH) levels were associated with the symptoms of joint pain in OA (12-14), in patients with the DISH syndrome (15), and in patients with acromegalic arthropathy (13). Hypertrophic osteoarthropathy responded clinically only to drugs which inhibited GH (14). Thus, if one includes in the criteria for OA, biochemical markers of disease, in addition to x-ray changes, a more complete definition of the OA process emerges. Previously, we showed that symptomatic OA patients had elevated serum GH levels and reduced serum insulin-like growth factor-I (IGF-1) levels, whereas patients without clinical symptoms showed normal serum levels of GH despite the presence of radiographic changes consistent with OA (16). Thus, serum GH, IGF-1 and insulin levels appeared to return to normal serum values when patients with OA became asymptomatic suggesting a role for GH and these other components of the GH pathway in OA disease progression. These hormones would be expected to play an especially prominent role in the inflammatory component of the disease which contributes significantly to the symptoms of the disease.

In homeostasis, GH and IGF-1 exist in a self-regulatory reciprocal relationship. GH stimulates liver IGF-1 synthesis, and in turn, IGF-1 stimulates various pathways of cartilage metabolism including proteoglycan biosynthesis (17). However, in OA this mechanism fails to function normally. Hochberg *et al*. (18) reviewed the initiation and perpetuation of the GH pathway beginning with the formation of a hormone-receptor complex resulting in "episodic" secretion of GH. The cell membrane then becomes refractory. GH binding to its receptor decreases until recycling of GH receptors results in GH receptor re-utilization. This cycle harmonizes with the secretory pattern of GH from the pituitary gland. The "episodic" nature of GH release or secretion is necessary to permit target organs to rest between episodes of GH release or secretion to inhibit GH shutdown or GH receptor refractoriness. Since GH is a poly-functional hormone, chronic exposure to excessive levels of GH may result in inflammation, overgrowth of specific tissues or modification of hormonal response to other agents. Acute exposure to excessive doses of GH may also be deleterious by suppressing the release or synthesis of IGF-1 by the liver. A key point is the understanding of how individuals control the excess serum GH levels since evidently as much GH is degraded as is synthesized to maintain homeostasis in the normal person.

In order to better understand the relationship between GH and OA, a mechanism was sought which could explain how the individual might be protected from large amounts of "toxic" GH. We hypothesized that excess GH might be removed from the extracellular serum compartment to an intracellular depot. The erythrocyte might serve such a function. To study this possibility, red blood cell (RBC) hemolysates were assayed for GH in patients with OA and also in suitable control individuals (table 1).

Sandulyak (19) had previously demonstrated that red cells could serve as depots for hormones other than GH, e.g. insulin. GH was found in RBC hemolysates using the method of Matuliavichius *et al*. (20) (table 1). However, not all persons studied (control or OA patients), had GH levels in RBC hemolysates that were in the detectable range (20).

Table 1. Concurrent Intra-Erythrocyte GH (GH-RBC) and Plasma GH (GH-P) Levels in Normal Subjects (N) and Osteoarthritic (OA) Male Patients

N/OA	Number	Age (yrs)[a]	GH-RBC (ng ml $^{-1}$)[a]	GH-P (ng ml^{-1})[a]
N	8	63 ± 8	1.7 ± 0.5	0.8 ± 0.3
OA	5	65 ± 7	3.8 ± 1.5	1.3 ± 0.3
		P > 0.05[3]	P < 0.003	P < 0.007
N	13	52 ± 15	†	0.8 ± 0.1
OA	4	56 ± 8	†	1.1 ± 0.2
		P > 0.05		P < 0.001

[a] mean ± S.D. , †GH levels below 0.4 ng ml^{-1}, [3]P-values were calculated by Student's T-test

Table 2. Concurrent Intra-Erythrocyte GH (GH-RBC) and Plasma GH (GH-P) Levels in Normal Subjects (N) and Osteoarthritic (OA) Female Patients

N/OA	Number	Age (yrs)[a]	GH-RBC (ng ml $^{-1}$)[a]	GH-P (ng ml^{-1})[a]
N	17	35 ± 12	1.8 ± 0.6	1.0 ± 0.5
OA	13	65 ± 10	2.1 ± 1.0	1.5 ± 0.7
		P < 0.0001[3]	P > 0.05	P < 0.0004
N	12	43 ± 20	†	1.2 ± 0.4
OA	5	68 ± 6	†	1.4 ± 0.4
		P < 0.02		P > 0.05

[a] mean ± S.D., †GH levels below 0.4 ng ml^{-1}, [3] P-values were calculated by Student's T-test

Thus, in these cases, patients were subdivided based on GH levels in the RBC. In males matched for age, where RBC GH levels could be detected, RBC GH levels from patients with OA were greater than in the control group (table 1). In another male subset group matched for age where RBC GH levels were below the detection levels (i.e., 0.4 ng ml^{-1}), plasma GH levels were higher in the OA group than were in the control group (table 1). Among female subjects tested in this series, the OA patients were older than their non-OA counterparts (table 2).

However, since serum GH levels were not age-dependent (see Tables 3 and 4, below), a comparison of concurrent intra-erythrocyte GH and plasma GH levels was also assessed (table 2). The plasma GH levels of OA patients was greater than that of the normal group, but the RBC GH levels, while higher in the OA group than in the normal group, did not reach statistical significance (table 2).

Curiously, among the females tested, where the RBC GH levels were below the limits of detection, the plasma GH level among the two groups was not different. Taken together, these results suggest that in some OA patients, RBC GH levels as well as plasma GH levels exceed the GH levels in the normal control group.

Taken together, we speculate that storage of "excess" GH in RBC may reduce the inflammatory or otherwise "toxic" effects of GH. In addition, RBCs containing high levels of GH circulate to the liver where GH is metabolized. In turn, RBCs also transport GH to tissues where GH exerts its characteristic metabolic actions.

To study the interactive regulatory mechanisms governing the GH/IGF-1 axis we turned to an evaluation of concurrent levels of GH and IGF-1 in normal controls and in patients with OA. Because of the disparity in the number of subjects in each patient subgroup, the results of this analysis are expressed as the average serum GH and IGF-1 levels with the range of the values obtained shown in parenthesis (table 3 and table 4). A cohort of normal individuals served not only as a control population for the patients with OA, but were also useful in that they helped establish baseline standards for the occurrence of spontaneous hypersecretory episodic levels of GH and IGF-1. The secretion of GH and IGF-1 differed among men and women. GH hypersecretors are more common among adult females, but the incidence of GH hypersecretion diminished with age in adult women. Normal GH levels, however, did not change with age either in men or women, while IGF-1 levels did diminish with age possibly as a result of changes that affect the liver. For purposes of this study, GH hypersecretion was set as three-times normal values. In patients with OA, the incidence of hypersecretory episodes decreased from 10% to 4% in men and from 18% to 3% in women. IGF-1 levels, on the other hand, were always lower in patients with OA and confirms previously published data (20). An additional confounding variable was also addressed. To explain the findings that x-ray changes compatible with OA occur in normal individuals, we considered that normal eating induces changes in GH, IGF-1 and insulin that are subtle and perceived as changes in normal stress and dietary changes. Gradually, however, x-ray changes in OA are found without any perceived clinical symptoms at all. In this respect, OA may be considered a metabolic disorder similar to diabetes mellitus with the exception that in OA, GH is the driving force instead of insulin.

5. CARTILAGE MATRIX SYNTHESIS AND DEGRADATION IN OA

How is cartilage injury related to potential systemic disturbances in the pathogenesis and perpetuation of the OA lesion? A principal tenet holds that initiation of cartilage damage results from a metabolic imbalance between chondrocyte anabolic and catabolic factors. Thus, metabolic disturbances may be expected to

Table 3. Serum GH and IGF-1 Levels in Normal (N) and Osteoarthritis (OA) Females

Subgroup	Number (%)	Age (yrs)	GH (ng ml^{-1})	IGF-1 (ng ml^{-1})
N				
Episodic	4 (27)	16	6.8 (3.0 - 11.0)	40.6 (28.2 - 53.3)
Non-episodic	11 (73)	17	0.8 (0.7 - 1.3)	36.5 (26.8 - 50.7)
N				
Episodic	23 (32)	28	5.8 (3.1 - 11.7)	26.8 (16.6 - 37.3)
Non-episodic	49 (68)	30	1.0 (0.4 - 1.9)	25.2 (13.4 - 72.5)
N				
Episodic	7 (18)	54	5.6 (2.9 - 14.7)	15.1 (9.4 - 25.4)
Non-Episodic	31 (82)	58	1.0 (0.4 - 2.2)	16.2 (8.1 - 28.8)
OA				
Episodic	1 (3)	66	4.1	14.3
Non-Episodic	30 (97)	67	1.5 (0.8 - 2.6)	12.1 (7.2 - 19.0)

Averages and range of values are shown for each group. In order for GH to be categorized as episodic, the serum GH level must be greater than three-times the average value of GH in normal persons.

Table 4. Serum GH and IGF-1 Levels in Normal (N) and Osteoarthritis (OA) Males

Subgroup	**Number (%)**	**Age (yrs)**	**GH (ng ml^{-1})**	**IGF-1 (ng ml^{-1})**
N				
Episodic	2 (18)	19	4.3 (3.9 - 4.8)	30.3 (21.3 - 39.3)
Non-episodic	9 (82)	17	0.7 (0.4 - 1.1)	55.2 (33.0 - 76.8)
N				
Episodic	5 (10)	28	6.6 (2.7 - 11.4)	29.6 (19.8 - 44.5)
Non-episodic	43 (90)	32	0.8 (0.4 - 1.4)	27.3 (14.0 - 39.9)
N				
Episodic	3 (10)	59	2.8 (2.4 - 3.5)	23.2 (8.9 - 24.8)
Non-episodic	27 (90)	60	0.8 (0.4 - 1.4)	20.1 (10.0 - 32.1)
OA				
Episodic	1 (4)	70	3.6	15.2
Non-episodic	22 (96)	63	1.2 (0.8 - 1.4)	15.2 (7.4 - 20.0)

Average and range of values are shown for each group. In order for GH to be categorized as episodic, the serum GH level must be greater than three-times the average value of GH in normal persons.

participate in the upregulation of enzyme pathways which accelerate the destruction of cartilage extracellular matrix components or induce chondrocyte apoptosis which compromises intrinsic attempts at cartilage repair. Recent evidence from animal models of OA (21) now implicate the synovium as playing an early role in the initiation (21,22) and perpetuation of cartilage damage (23). Thus, the avascular and aneural character of cartilage, previously implied to shield cartilage from the potential deleterious effects of systemic disturbances may have to be reconsidered as a result of synoviocyte activation characterized by transcriptional activation of cytokine (24) and metalloproteinase (MMP) genes (22). Cytokines such as interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-alpha) have been implicated in the transcriptional upregulation of several of the MMPs including MMP-3 [stromelysin] (25-27), MMP-1 [collagenase-1] (28,29), MMP-9 (30), MMP-13 [collagenase-3] (31-33), MMP-8 [neutrophil collagenase] (34,35) and an enzyme activity, namely, aggrecanase (36,37). which degrades aggrecan in the intraglobular domain but at a site different from that of MMP-3 (38,39). In addition to aggrecan and the interstitial collagen isotypes, types I, II and III collagens these MMPs have broad substrate reactivity against other extracellular matrix proteins of articular cartilage (40). The significance of the upregulation of the MMPs by Il-1 was further established by the ability of IL-1 receptor antagonist to inhibit the transcription of MMPs and to ablate experimental arthritis (41,42). Elevated levels of TNF-alpha have also been found in the synovial fluid of early osteoarthritic canine lesions (26). Recent information has also emerged which implicates a membrane-bound class of MMPs (MT-MMPs) in cartilage degradation (43,44). The transcription of MT1-MMP was upregulated by IL-1 and TNF-alpha (43). MT1-MMP cleaved aggrecan at the "aggrecanase" and the other MMP sites (44). MT1-MMP was shown to activate MMP-2 (gelatinase) which has a wide range of substrate specificity in articular cartilage (43).

While the upregulation of MMPs by cytokines is well established, the control over the activity of MMPs and degradation of cartilage proteins appears to rest in the apparent imbalance between MMPs and their endogenous inhibitors, the tissue inhibitors of metalloproteinases [TIMPs] (45-47). The mechanism by which TIMPs inhibit MMPs has now been established (48). The degradation of cartilage substrates in OA would be characterized as an

imbalance between the upregulatory events that govern MMPs and TIMPs (46) where MMP gene expression was markedly elevated at the expense of TIMPs.

Because many of the studies to determine substrate degradation patterns were conducted with recombinant MMPs and purified proteins *in vitro*, it has been crucial to determine which, if any, of these MMPs are active in the synovial joints of OA patients and what are the characteristics of the substrate degradation fragments found in the synovial fluid and cartilage. Monoclonal antibodies produced against specific neoepitopes of aggrecan and Type II collagen have been instrumental in assigning significance to the types and role of the various MMPs in question (49). Taken together, the evidence indicates that substrate-specific degradation *in vitro* results in protein fragments which can now be also identified in OA synovial fluid (50) making the case much stronger that these MMPs play a critical role in OA cartilage degeneration. Specific Type II collagen fragmentation and loss from articular cartilage also correlated strongly with the severity of OA cartilage lesions (51).

5.1. Cytokine regulation

The effectiveness of cytokines as up-regulators of MMP genes would be dependent not only on the cytokine levels (24, 26), but also on the relative sensitivity of refractoriness of the chondrocytes to the presence of these cytokines. This element of the cytokine pathway is likely to be controlled by increased receptors for IL-1 (IL-1r) in human chondrocytes and TNF-alpha in human synoviocytes derived from OA cartilage (25, 52-55). The synergistic action of IL-1 beta and TNF-alpha may also modulate TNF-alpha receptors (53).

5.2. Compensatory matrix biosynthesis

Compensatory biosynthesis of cartilage extracellular matrix components may also be compromised in OA. In short-term explant cultures of human OA cartilage obtained at joint replacement surgery, the amount of aggrecan synthesized was reduced when compared to the synthesis of the small leucine-rich proteoglycans, decorin and biglycan (56). Studies of the endogenous biglycan and decorin levels in OA cartilage, however, have provided conflicting results. Several studies which measured cartilage decorin content (57) or cartilage decorin and biglycan mRNA levels (58) sustained the biosynthesis results (56). Another study which employed monoclonal antibodies found no differences in cartilage decorin and biglycan content when OA and aged non-arthritic cartilage were compared (59). Potential repair of early cartilage lesions may be impaired by increased amounts of decorin owing to the reported anti-adhesive properties of decorin (60, 61).

6. RELATIONSHIP OF CARTILAGE CHANGES IN OA TO METABOLIC DISTURBANCES

Cartilage matrix synthesis is also compromised by the presence of IL-1 beta (62, 63). Furthermore, IL-1 beta was shown to alter IGF-1 gene expression (64) which may also modify proteoglycan gene expression. Abortive chondrocyte proliferation typical of early chondrocyte responses in OA could also result from chondrocyte unresponsiveness to IGF-1 (65,66) and the reduction of chondrocyte content from those systemic alterations in metabolic pathways (such as those that induce nitric oxide synthase), then in turn, accelerate apoptosis in articular cartilage (67, 68). The IGF-1 response may have evolved from IGF-1/IGF-1 receptor interactions which become abnormal in OA due to the presence of increased amounts of IGF-1 binding proteins (65, 66) which act as IGF-1 agonists in this process. The serum levels of the GH and IGF-1 in patients with OA are abnormal and the normal reciprocal relationship of the GH/IGF-1 axis is skewed. Thus, decreased levels of peripheral IGF-1 dampened by elevated GH levels, in addition to impaired IGF-1/IGF-1 receptor interactions would further compromise the cartilage repair process.

That restoration of normal IGF-1 levels could play an important role in maintaining cartilage homeostasis in early OA was suggested by studies in a canine model of OA induced by surgical transection of the anterior cruciate ligament (69). Six weeks after induction of OA, dogs treated with both IGF-1 and pentosan polysulfate (PPS) demonstrated a gross cartilage appearance that was almost normal. However, animals treated with either PPS or IGF-1 alone showed gross signs of OA pathology. Animals treated with IGF-1 and PPS, showed less total and active MMPs and total collagenase in OA cartilage. TIMP levels approaching normal were found in OA cartilage after these treatments. These data also suggested that, at least in this OA animal model, chondrocytes maintained their responsiveness to IGF-1. Thus, abortive repair of cartilage lesions seen in human OA pathological samples could result, at least in part, from impaired IGF-1 synthesis and function. Understanding mechanisms required to restore chondrocyte responsiveness to IGF-1 in human OA cartilage could provide an additional avenue for therapy designed to support cartilage compensatory matrix biosynthesis.

7. CONCLUSIONS

The results of longitudinal studies of serum GH and IGF-1 in patients with OA during periods of clinical activity and during remission strongly implicate systemic disturbances in the pathogenesis and progression of OA. The perpetuation of pathological processes involving chondrocyte and synovial tissue activation pathways appear to favor cartilage degradation which may be further exacerbated by normal mechanical forces acting on a compromised cartilage extracellular matrix. Thus, the pathogenesis and progression of OA should no longer be interpreted as simply a result of focal synovial joint pathology. In its place, it is proposed that OA be considered a metabolic disturbance with characteristic specific alterations in cartilage matrix biosynthesis and degradation accompanied by a robust remodeling of the synovial joint architecture including the formation of new bone (i.e. osteophytes). The evidence also strongly indicate that the serum acute phase reactant pattern in patients with symptomatic OA are quite similar to those patterns seen in patients with inflammatory arthritis.

Insights into the fundamental degradation and repair processes occurring in the OA joint will be gained when it is possible to decipher the complex interactions between GH, IGF-1 and other cytokines and growth factors in the peripheral circulation and how they interact with each other to cause destruction of cartilage and compromise repair of cartilage in the synovial joint.

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