RESEARCH ARTICLE

Soluble Mediators in Posttraumatic Wrist and Primary Knee Osteoarthritis

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Abstract

Background: New discoveries about the pathophysiology changed the concept that all forms of osteoarthritis are alike; this lead to the delineation of different phenotypes such as age, trauma or obese related forms. We aim to compare soluble mediator profiles in primary knee and posttraumatic wrist osteoarthritis. Based on the general faster progression rate of wrist osteoarthritis, we hypothesize a more inflammatory profile.

Methods: We collected synovial fluid from 20 primary osteoarthritic knee and 20 posttraumatic osteoarthritic wrist joints. 17 mediators were measured by multiplex enzyme-linked immunosorbent assay: chemokine ligand 5, interferon-γ, leukemia inhibitory factor, oncostatin-M, osteoprotegerin, tumor necrosis factor-α, vascular endothelial growth factor, interleukin (IL)-1α, IL-1β, IL-1 receptor antagonist, IL-4, IL-6, IL-7, IL-8, IL-10, IL-13 and IL-17.

Results: Ten mediators were higher in posttraumatic osteoarthritic synovial fluid: tumor necrosis factor-α (TNFα), IL-1α, IL-1RA, IL-6, IL-10, IL-17, oncostatin-M, interferon-γ, chemokine ligand 5 and leukemia inhibitory factor (P<0.001). IL-1ß, IL-4, IL-7 were not detected, TNFα was not detected in knee osteoarthritic synovial fluid. IL-8, IL-13, osteoprotegerin and vascular endothelial growth factor levels did not differ between the synovial fluid types.

Conclusions: In general wrist osteoarthritis seems characterized by a stronger inflammatory response than primary knee osteoarthritis. More pronounced inflammatory mediators might offer a paradigm for the faster progression of posttraumatic osteoarthritis. Increase of specific mediators could form a possible target for future mediator modulating therapy in wrist osteoarthritis.

Key words: Cytokines, Knee, Osteoarthritis, Posttraumatic, Wrist

Introduction

New discoveries about the pathophysiology have changed the concept that all forms of osteoarthritis are alike and share the same clinical and structural characteristics (1). This notion leads to the delineation of different clinical and structural phenotypes such as age, trauma or obesity dominated forms of the disease (2).

Wrist osteoarthritis is mainly posttraumatic and characterized by faster progression at a younger age when compared to primary forms of osteoarthritis (3, 4). Altered joint mechanics are recognized to be a driving force in wrist osteoarthritis. However, the concept of residual joint instability after joint trauma as the sole cause of wrist osteoarthritis seems insufficient as osteoarthritis develops even if reconstructive surgery successfully stabilizes the joint (5, 6). This suggests a role for anabolic and catabolic soluble mediators such as growth factors, cytokines, and chemokines from the time of the initial joint injury up to end stage osteoarthritis (5, 7, 8).

The aim of the study was to compare the soluble mediator profiles of posttraumatic wrist osteoarthritis to that in primary knee osteoarthritis. Based on the

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general faster progression rate of posttraumatic wrist osteoarthritis, we hypothesize a more inflammatory profile.

Materials and Methods

Patient characteristics

We collected synovial fluid from two groups of patients: posttraumatic wrist osteoarthritis samples (n=20) were obtained during various surgeries for end-stage radiocarpal osteoarthritis. Patients in this group had clinical symptoms and radiological changes consistent with advanced osteoarthritis of the radiocarpal joint. All of these patients had a history of wrist trauma. Primary kneeosteoarthritis (n=20) synovial fluid was acquired during total knee replacement due to endstage osteoarthritis. The American College of Rheumatology criteria for osteoarthritis were met by patients included in both groups (9). Exclusion criteria were infection, rheumatoid arthritis, and other forms of inflammatory arthritis.

In accordance with 'good use of redundant tissue for research' constructed by the Dutch Federation of Medical Research Societies, tissue samples were anonymized precluding use of patients' characteristics for detailed data analysis. Therefore, synovial fluid samples could not be matched for age, BMI or sex. Collection of synovial fluid was approved by the Medical Ethics Committee of our institution (12-223/C).

Sample collection

Knee synovial fluid was aspirated directly after opening of the joint capsule. Due to the low amount of synovial fluid in the wrist joint, samples were collected by pre-weighed, standard size, sterile gauze swabs. This technique allows collection of synovial fluid when the available quantity is low (8). Immediately after opening of the radiocarpal joint, a sample of synovial fluid was absorbed. The saturated swab was then placed in 500 µl HPE-0.1375% Tween buffer solution (Sanquin, Amsterdam, Netherlands). Both wrist and knee synovial fluid samples were vortexed prior to a two minute 3000 rounds per minute centrifuge cycle to spin down any cells or debris. Thereafter, the supernatant was stored at -80 °C until further analysis.

As we could not reliably determine the exact volume of the swabbed synovial fluid samples by their weight, all cytokine levels were normalized to their protein content.To quantify the protein levels, we performed a bicinchoninic acid protein assay (Thermo scientific, #23227,Rockford, USA) according to the manufacturer's protocol. In short, a standard curve was made using bovine serum albumin. Pretreated synovial fluid samples were incubated for 30 minutes at 37°C with color reagent A+B and measured at 540 nm. The protein concentration was calculated using the standard curve and expressed as micrograms per milliliter.

Multiplex enzyme-linked immunosorbent assay

We measured 17 mediators: interleukin (IL)-1α and β, IL-1 receptor antagonist (RA), IL-4, IL-6, IL-7, IL-8, IL-10, IL-13, IL-17, chemokine ligand five (CCL5), interferon (IFN)-γ, leukemia inhibitory factor (LIF), oncostatin-M (OSM), osteoprotegerin (OPG), tumor necrosis factor (TNF)-α and vascular endothelial growth factor (VEGF). We based our selection on findings in rheumatoid arthritis, osteoarthritis and the availability of mediator modulating therapies (7, 10, 11). Samples were analyzed using a multiplex enzyme-linked immunosorbent assay, as previously described by de Jager et al. (12, 13). In short, we pretreated 200 µL of each of the synovial fluids with buffer solution samples with 10 µl hyaluronidase (Sigma, St, Louis, MO; 10 mg/mL) for 30 min at 37°C. Samples were spun down in a X-column (Costar 8169; Corning, Amsterdam, Netherlands). Finally, 5µL rat-mouse serum was added to bind any residual interfering antibodies. Pretreated samples were incubated with precoatedcarboxylated beads (Luminex Corp, Austin, TX). Recombinant proteins were used to make a standard curve. Treated samples were incubated with the coupled beads. After incubation with the appropriate biotinylated antibodies, samples were thoroughly washed and incubated with streptavidin-PE for 10 minutes. After washing, samples were measured and analyzed using the Bio-Plex suspension system (Bio-Rad Laboratories, Hercules, CA) with Bio-Plex Manager software, version 5.0. All samples were measured in duplicate in the same plate. Concentrations of mediators in the synovial fluid were calculated using standard curves.

Statistical Analysis

Shapiro-Wilktest showed a predominantely non-Gaussian distribution of the data and cytokine levels were compared by Mann-Whitney U test. We determined and adjusted the level of significance (*P*) using the Bonferroni correction for multiple testing. A *P* value of <0.0033 was considered significant (i.e., adjusted *P* value cut off =.05/15=0.0033). All statistical analyses were conducted in Stata 13 (Stata Corp LP, College Station, TX). As cytokine levels were normalized to their protein content, we report normalized concentrations as 10-3picogram mediator/microgram protein/ml, median ± interquartile range.

Results

Out of the 17 measured soluble mediators, 10 were higher in posttraumatic wrist than in primary knee osteoarthritis: IL-1α, IL-1RA, IL-6, IL-10, IL-17, CCL5, IFNγ, LIF, OSM and TNFα (*P*<0.001).TNFα could not be detected in any of the primary knee osteoarthritis samples, whereas it was detectable in wrist synovial fluid. IL-1ß, IL-4 and IL-7 were not detected in either group; IL-8, IL-13, OPG and VEGF did not differ between the two groups (Table 1).

Discussion

New discoveries about osteoarthritis pathophysiology lead to the delineation of different phenotypes such as age, trauma or obese related forms.The aim of the study was to compare soluble mediator profiles of posttraumatic wrist osteoarthritis and primary knee osteoarthritis. We noted distinct differences in concentration between posttraumatic wrist and primary knee osteoarthritis

Table 1. Concentration of mediators in synovial fluid of patients with primary knee and posttraumatic wrist osteoarthritis

Chondrodestructive	Wrist OA (median ± IqR)	Knee OA (median ± IqR)	*P* value
IL-1a	27 (14-51)	0.91 (0-1.4)	<0.001
IL-1b	ND	ND	
IL-7	ND	ND	
IL-8	85 (51-162)	52 (34-71)	0.0397
IL-17	353 (213-570)	9.6 (3.8-16)	<0.001
CCL5	3554 (2170-5535)	209 (86-395)	<0.001
IFN-g	146 (83-183)	5.6 (4.7-10)	<0.001
LIF	431 (183-661)	11 (5.9-21)	<0.001
OSM	40 (17-61)	0.49 (0-5.0)	<0.001
TNF-a	1.0 (0-3.6)	ND	0.0011
VEFG	131 (82-325)	216 (126-358)	0.0989
Chondroprotective			
IL-1RA	249 (155-390)	18 (15-25)	<0.001
IL-4	ND	ND	
IL-10	259 (217-473)	39 (35-56)	<0.001
IL-13	ND	0 (0-0.73)	0.009
OPG	721 (391-1215)	2137 (802-6575)	0.0045
Chondromodulatory			
IL-6	533 (404-713)	39 (27-83)	<0.001

OA = osteoarthritis; IqR = interquartile range
Concentrations are in 10^{-3}picogram mediator / microgram protein / ml

mediators.

Several potential shortcomings of the study should be kept in mind. Firstly, to allow comparison of samples, we normalized mediator levels to their protein concentrations. Synovial fluids from different joints were previously shown to contain similar levels of serum proteins in rabbits (14). Although it cannot be excluded that serum protein levels change when joints are inflamed or that the similarity between joints may not hold true for humans, this was the most appropriate way to normalize between samples. Secondly, different joints might have different inflammatory responses; previous study described that inflammation as characterized by macrophage and T-cell number and Il-6 production was not dependent on joint type in the human patient (15). Thirdly, our correcting for multiple testing might have been too stringent and this study may not be adequately powered to detect smaller differences.

Although some mediators are protective, such as Il-10 and IL-1RA, in general, posttraumatic wrist osteoarthritis seemed characterized by a stronger inflammatory response as proinflammatory mediators (IL-1α, IL-6, IL-17, CCL5, IFNγ, LIF, OSM and TNFα) were higher. Despite the promising protective effects of IL-13 and IL-4, our data confirm the existing literature indicating a limited role for those cytokines in end stage osteoarthritis as IL-4 could not be detected and only low levels of IL-13 were found in knee osteoarthritis (11, 16, 17). IL-1ß and TNFα have been indicated as prominent inflammatory mediators associated with cartilage degeneration, bone changes and synovial inflammation (1, 18). IL-1 family cytokines seem to play an important role in the period directly following joint trauma as increased IL-1 expression has been documented after mechanical joint injury and correlates with the severity of cartilage damage (19). In in vivo animal models of osteoarthritis blocking IL-1ß and TNFα gave promising results; however, this could not be validated in clinical studies (20). In both the literature and our study, IL-1ß and TNFα were not detectable or were detected at low levels in synovial

fluid from patients with end stage posttraumatic wrist as well as primary knee osteoarthritis (17). Our results, likewise, do not support a prominent role for IL-1ß and TNFα in end stage osteoarthritis.

For this study we selected two very different groups of patients with osteoarthritis and demonstrated a pronounced differences in cytokine levels. This possible difference might be related to joint type (knee vs. wrist), and also to differences in pathology (primary vs. posttraumatic osteoarthritis). Previous study described that inflammation as characterized by macrophage and T-cell number and Il-6 production was not dependent on joint type in the human patient (15). We speculate that the more pronounced inflammatory response in the wrist samples is related to the posttraumatic origin and that increased inflammation might offer a paradigm for the faster progression of posttraumatic osteoarthritis compared to primary osteoarthritis. Future research should focus on (1) testing mediators in other joints prone to posttraumatic osteoarthritis (e.g., shoulder and ankle) and (2) comparing primary and posttraumatic osteoarthritis within the same joint.

Lack of in-depth understanding of phenotype pathogenesis restricts further development of diagnosis, treatment, and monitoring of the many forms of osteoarthritis. Our study is the first to compare cytokine levels and to show differences between subgroups of osteoarthritis. This study contributes to our knowledge and vision on the role of cytokines in the pathogenesis of osteoarthritis and can help direct further research to markers and treatments for osteoarthritis. Many new pharmacological approaches in the management of osteoarthritis are under development and mediator modulation could be a promising therapy in wrist osteoarthritis (1, 21, 22). Growing knowledge of the pathogenetic mechanisms involved in osteoarthritis improves understanding and will allow specific targeted treatment in those with different subsets of the disease.

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References

1. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheumatol. 2010; 7:33-42.
2. Bijlsma JW, Berenbaum F, Lafeber FP. Osteoarthritis: an update with relevance for clinical practice. Lancet. 2011;377:2115-26.
3. Marsh JL, Weigel DP, Dirschl DR. Tibial plafond fractures. How do these ankles function over time? J Bone Joint Surg Am. 2003; 85-A:287-95.
4. Knirk JL, Jupiter JB. Intra-articular fractures of the distal end of the radius in young adults. J Bone Joint Surg Am. 1986; 68:647-59.
5. Lotz MK, Kraus VB. New developments in osteoarthritis. Posttraumatic osteoarthritis: pathogenesis and pharmacological treatment options. Arthritis Res Ther. 2010; 12:211.
6. Marsh JL, Buckwalter J, Gelberman R, Dirschl D, Olson S, Brown T, et al. Articular fractures: does an anatomic reduction really change the result? J Bone Joint Surg Am. 2002;84-A:1259-71.
7. Rutgers M, Saris DB, Yang KG, Dhert WJ, Creemers LB. Joint injury and osteoarthritis: soluble mediators in the course and treatment of cartilage pathology. Immunotherapy. 2009; 1:435-45.
8. Teunis T, Beekhuizen M, Kon M, Creemers LB, Schuurman AH, van Minnen LP. Inflammatory mediators in posttraumatic radiocarpal osteoarthritis. J Hand Surg Am. 2013; 38:1735-40.
9. Altman R, Alarcon G, Appelrouth D, Bloch D, Borenstein D, Brandt K, et al. The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hand. Arthritis Rheum. 1990; 33:1601-10.
10. Goldring SR, Goldring MB. The role of cytokines in cartilage matrix degeneration in osteoarthritis. Clin Orthop Relat Res. 2004; 427:27-36.
11. Fernandes JC, Martel-Pelletier J, Pelletier JP. The role of cytokines in osteoarthritis pathophysiology. Biorheology. 2002; 39:237-46.
12. de Jager W, Prakken B, Rijkers GT. Cytokine multiplex immunoassay: methodology and (clinical) applications. Methods Mol Biol. 2009; 514:119-33.
13. de Jager W, Prakken BJ, Bijlsma JW, Kuis W, Rijkers GT. Improved multiplex immunoassay performance in human plasma and synovial fluid following removal

of interfering heterophilic antibodies. J Immunol Methods. 2005; 300:124-35.
14. Knox P, Levick JR, McDonald JN. Synovial fluid--its mass, macromolecular content and pressure in major limb joints of the rabbit. Q J Exp Physiol. 1988;73:33-45.
15. Kraan MC, Reece RJ, Smeets TJ, Veale DJ, Emery P, Tak PP. Comparison of synovial tissues from the knee joints and the small joints of rheumatoid arthritis patients: Implications for pathogenesis and evaluation of treatment. Arthritis Rheum. 2002; 46:2034-8.
16. Rachakonda PS, Rai MF, Manning K, Schmidt MF. Expression of canine interleukin-4 in canine chondrocytes inhibits inflammatory cascade through STAT6. Cytokine. 2008; 44:179-84.
17. Beekhuizen M, Gierman LM, van Spil WE, Van Osch GJ, Huizinga TW, Saris DB, et al. An explorative study comparing levels of soluble mediators in control and osteoarthritic synovial fluid. Osteoarthritis Cartilage. 2013; 21:918-22.
18. Blom AB, van der Kraan PM, van den Berg WB. Cytokine targeting in osteoarthritis. Curr Drug Targets. 2007; 8:283-92.
19. Marks PH, Donaldson ML. Inflammatory cytokine profiles associated with chondral damage in the anterior cruciate ligament-deficient knee. Arthroscopy. 2005;21:1342-7.
20. Burger D, Dayer JM, Palmer G, Gabay C. Is IL-1 a good therapeutic target in the treatment of arthritis? Best Pract Res Clin Rheumatol. 2006;20:879-96.
21. Schnitzer TJ. New pharmacologic approaches in the management of osteoarthritis. Arthritis Care Res (Hoboken). 2010;62:1174-80.
22. Calich AL, Domiciano DS, Fuller R. Osteoarthritis: can anti-cytokine therapy play a role in treatment? Clin Rheumatol. 2010;29:451-5.