Cytokine 115 (2019) 45–49

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Short communication

Muscular-based and patient-reported outcomes differentially associate with circulating superoxide dismutases and cytokines in knee osteoarthritis

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ARTICLE INFO

Keywords:
Cytokines
Superoxide dismutase
Knee osteoarthritis
Clinical outcomes

ABSTRACT

Muscular (i.e., quadriceps) weakness contributes to disease progression and precedes the appearance of patient-reported symptoms, such as pain and perceived physical dysfunction, in knee osteoarthritis (OA). It is unknown, however, if muscular-based and patient-reported outcomes differentially associate with systemic biomarkers reflective of the local mediators in knee OA. The purpose of this study was to identify if muscular-based and patient-reported outcomes differentially associate with circulating superoxide dismutase (SOD) and cytokines in knee OA. Subjects (n = 29) with pain, muscular weakness, and radiographic evidence (Kellgren-Lawrence grade ≥2) of knee OA in the involved (INV) leg were included in this study. Serum Cu/Zn and Mn SOD and cytokine concentrations were measured in fasting blood samples. Pain and physical dysfunction were subjectively assessed and muscle strength (i.e., peak isometric force and torque, and peak isokinetic-concentric knee-extension and -flexion torques) was determined unilaterally in the INV and non-involved (NI) legs. Peak isometric and peak isokinetic-concentric knee-flexion torques in the INV leg correlated with serum Cu/Zn SOD (both $p < 0.05$). Peak isometric force and torque and peak isokinetic-concentric knee-extension and -flexion torques in the INV leg correlated with serum Mn SOD (all $p < 0.05$). Pain and dysfunction inversely associated with serum IL-1β, IL-4, IL-5, IL-12, IL-13, and/or IFN-γ ($p < 0.05$). Neither SOD associated with pain or dysfunction, and none of the cytokines associated with muscular-based outcomes. We conclude that common outcome measures used in the clinical evaluation of OA differentially associate with circulating SOD and cytokines.

1. Introduction

Failure to identify knee osteoarthritis (OA) patients at risk of a faster rate in disease progression continues to challenge our prognostic efforts, hinder patient care, and increase health care costs. Muscular (i.e., quadriceps) weakness is an early clinical observation in knee OA that contributes to disease progression and precedes the appearance of patient-reported symptoms [1], such as pain and perceived physical dysfunction. The sequential manifestation of disease-related symptoms is temporally dependent on local degenerative changes to the underlying articular cartilage, synovial tissue, bone, menisci, and ligaments. In an attempt to assist in the clinical decision process and improve patient care, there has been increasing interest to identify biomarkers in the blood that reflect local mediators of joint deterioration [2,3].

Superoxide dismutase (SOD) is an endogenous antioxidant enzyme that converts superoxide to hydrogen peroxide. In osteoarthritic cartilage and other localized cells from an osteoarthritic joint, data suggest an increase in superoxide and a down-regulation of SOD [4]. The decrease or down-regulation in SOD increases the susceptibility of oxidative stress-induced damage and the subsequent pathogenesis of OA [5], and in experimental mice, impairs skeletal muscle function [6]. Despite its regulatory influence on the disease pathogenesis, skeletal muscle function, and localized decrease with disease progression, it is unknown if circulating SOD associates with clinical symptoms reflective of disease progression in knee OA.

Among other cytokines and cytokine receptors, tumor necrosis

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https://doi.org/10.1016/j.cyto.2018.11.034
Received 5 September 2018; Received in revised form 12 November 2018; Accepted 29 November 2018
Available online 08 January 2019
1043-4666/

factor (TNF)-α and interleukin (IL)-1β are quintessential pro-inflammatory cytokines that facilitate the development and progression of OA by mediating cartilage degradation. In humans, serum TNF-α and IL-6 concentrations associate with narrowing of the knee joint space [7], suggesting cartilage loss associates with a systemic increase in pro-inflammatory cytokines. Furthermore, TNF-α and IL-1β induce IL-10, which is an anti-inflammatory cytokine that reciprocally inhibits the production of pro-inflammatory cytokines and locally upregulated in osteoarthritic cartilage and synovium [8]. In contrast to local increases, circulating IL-10 concentrations decrease in subjects with knee OA compared to controls [8], and data from our lab indicates that other cytokine (i.e., IL-5, IL-6, IL-12, IL-13, and TNF-α) concentrations in the blood decrease with advanced knee OA [9].

It is unknown if clinical outcomes that display temporally disparate rates of appearance during disease progression differentially associate with systemic biomarkers reflective of local mediators in knee OA. Therefore, we sought to identify if muscular-based and patient-reported outcomes differentially associate with circulating SOD (i.e., Cu/Zn and Mn) and cytokines in subjects with knee OA. We hypothesized that muscular-based outcomes correlate with circulating SOD, while patient-reported outcomes inversely correlate with circulating cytokines in knee OA subjects.

2. Methods

To address our hypothesis, modestly active (minimum of 30 min of continuous exercise or physical exertion 3 times per week during the previous year) subjects older than 18 but younger than 60 years of age were initially recruited and consented to study participation. Subjects were excluded from participation if: they had a recent (within 2 years) surgery on the involved (INV, symptomatic knee) or non-involved (NI, non-symptomatic knee) leg, reported known history of any disease or condition requiring medical attention or treatment (including but not limited to corticosteroid injections), pregnant, using a daily dietary supplement or vitamin during the previous year, morbidly obese (body mass index > 40 kg/m^2), current tobacco users, or scheduled to undergo total knee arthroplasty (TKA). Subjects were informed of and provided written and verbal consent to the protocol and procedures. The Central Region Institutional Review Board at Intermountain Healthcare (Salt Lake City, UT USA) approved this study. Please note, this communication provides a portion of the baseline data from a randomized, double-blind, placebo controlled study that will be presented in a later manuscript.

2.1. Eligibility screening

Following consent, each subject (n = 29 (f/m, 16/13), age, 49 (2) y; height, 169 (2) cm; body mass, 92.3 (3.6) kg; body mass index, 32.2 (1.1) kg/m^2) provided one fasting blood sample during eligibility screening. Subjects with hypo- (total calcium < 8.4 mg/dL) or hyper-calcemia (total calcium > 10.4 mg/dL), hypo-(parathyroid hormone < 12 pg/mL) or hyper-parathyroidism (parathyroid hormone > 72 pg/mL), elevated rheumatoid factor (> 15 IU/mL), or increased uric acid (females > 7.5 mg/dL; males > 8.5 mg/dL) were excluded from participation.

Subjects were also excluded from participation if: (1) the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain score was < 2 on any of the five questions in its subsection, (2) there was no evidence of muscular weakness (i.e., deficit in peak isokinetic-concentric knee extension or flexion torque at 60°/s) in the INV compared to the NI leg, and (3) a Kellgren-Lawrence grade < 2 was scored in the INV knee [10]. Therefore, this study consisted of subjects with knee pain, muscular weakness, and radiographic evidence of OA in the INV leg.

2.2. Study design and protocol

Following screening, each subject reported to the Physiology Research Laboratory at The Orthopedic Specialty Hospital (Murray, UT USA). At this visit, each subject provided a fasting blood draw sample, completed the patient-reported survey, and performed the muscular-based strength testing procedures.

2.3. Analytical procedures

2.3.1. Serum inflammatory biomarkers and superoxide dismutases

Serum cytokine and soluble cytokine receptor concentrations (pg/mL) were determined using the multiplex technology of Luminex (MAGPix; Austin, TX USA) with high sensitivity (EMD Millipore; Billerica, MA USA). Serum SOD (Mn and Cu/Zn; ng/mL) concentrations (ng/mL) were determined using the multiplex technology of Luminex.

2.3.2. Muscular-based outcomes

2.3.2.1. Single-leg peak isometric force. Single- (i.e., NI versus INV) leg strength testing was performed first on a horizontal Plyo-Press (Athletic Republic, Park City, UT USA) with mounted force plate (Advanced Mechanical Technology, Watertown, MA, USA), and second, on an upright Biodex S4 (Shirley, NY USA), as previously described [11,12]. In brief, subjects were placed on a horizontal Plyo-Press sled with the sled and foot placement adjusted for each subject to align the knee and hip joint flexion angles to 90°. In this position, hip- and knee-extension peak isometric force (N and N/kg) production was tested. The first single-leg peak isometric contraction was performed on the NI limb and the second contraction was performed on the INV limb. This sequencing of testing (i.e., NI leg first and INV leg second) was performed in duplicate, and thereby, allowing for two peak isometric contractions performed on each leg separately. The rationale for performing the peak isometric contraction on the NI limb first was to allow each subject to experience the testing protocol on the asymptomatic leg prior to testing the symptomatic leg. Each isometric contraction was 3-seconds in duration and contractions were separated by 1-minute of rest. Subjects were verbally instructed and strongly encouraged to exert maximal force (i.e., hip- and knee-extension) force against the mounted force plate on the Plyo-Press. Peak isometric force was defined as the highest resultant force produced by each leg separately.

2.3.2.2. Single-leg peak isometric torque. Following the isometric testing on the horizontal Plyo-press, each subject then performed single-leg peak isometric torque (Nm) strength testing on a Biodex S4. Subjects were seated in an upright (90° hip flexion) position with the chair and dynamometer adjusted to align the lateral condyle of the femur to the center of the dynamometer shaft. Peak concentric knee extension-isometric contractions were performed in duplicate on each leg separately at 60° of knee flexion starting with the NI leg. Each contraction was 5-seconds in duration and separated by 1-minute of rest. Following the completion of the testing on the NI leg, subjects then repeated the testing procedures on the INV leg. Similar to the testing described above, the rationale for performing the isometric contractions on the NI limb first was to allow each subject to experience the testing protocol on the asymptomatic leg prior to testing the symptomatic leg. Peak-concentric knee extension isometric torque was defined as the highest torque produced by the NI and INV legs separately.

2.3.2.3. Single-leg peak isokinetic-concentric knee-extension and -flexion torques. After completing the peak isometric force and torque testing, each subject then performed single-leg peak isokinetic-concentric knee-extension (Nm) and -flexion (Nm) contractions in the same upright position and Biodex S4 settings described above. Each subject performed 6 consecutive concentric knee extension-flexion contraction cycles at 60° per second through a full range of motion (90° of knee flexion to full extension) on the NI knee first. Following the

completion of the contractions on the NI knee, subjects then repeated the isokinetic testing protocol on the INV knee. Subjects were instructed and verbally encouraged to perform every contraction with maximal effort during all isometric and isokinetic testing procedures.

As anticipated, peak isometric force, isometric torque, and concentric isokinetic (knee-extension and -flexion) torques correlated in the NI (r = 0.55–0.85, p = 0.002 to < 0.001, n = 29; data not shown) and INV legs (r = 0.59–0.93, all $p \leq 0.001$, n = 29, data not shown) separately. Furthermore, peak isometric force, isometric torque, and concentric isokinetic torques (knee-extension and -flexion) correlated between the NI and INV legs (r = 0.82–0.92, all $p \leq 0.001$, n = 29, data not shown), collectively suggesting a positive relationship among the muscular-based outcomes within and between the asymptomatic and symptomatic legs.

2.3.3. Patient-reported outcomes

Subjects completed the pain (0–20; 0 no pain; 20 extreme pain) and physical function (0–68; 0, no physical impairment; 68, extreme physical impairment) subsections of the WOMAC questionnaire.

2.3.4. Radiographic evidence of knee OA

X-ray images were obtained on each knee in the anterior-posterior view at 45° of knee flexion. Knee joint space (mm) was analyzed using ImageJ software (National Institutes of Health). Severity of knee OA was classified according to the scoring criteria established by Kellgren and Lawrence [10]: 0, no osteophytes or joint-space narrowing; 1, questionable osteophyte indicating possible OA; 2, definite osteophyte, no joint-narrowing (compared to the NI knee) indicating mild OA (n = 3); 3, ≤50% joint-space narrowing (compared to the NI knee) indicating moderate OA (n = 18); and 4, > 50% joint-space narrowing (compared to the NI knee) indicating severe OA (n = 8).

2.4. Statistical analyses

Data were checked for normality prior to statistical analyses with a Shapiro-Wilk Test. Associations between variables were assessed with a Pearson Product Moment Linear correlation. Statistical significance between legs (NI vs INV) were assessed with a *t*-test (Supplemental Table). Significance was set at $p < 0.05$ and all statistical analyses were performed with SYSTAT (version 13.1, Chicago, IL, USA). Data presented as mean (SEM) unless noted otherwise.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.cyto.2018.11.034.

3. Results and discussion

Muscular weakness is a predominant impairment and precedes the development of localized pain and physical dysfunction in knee OA. In this investigation, we provide the first evidence that muscular-based outcomes associate with circulating Cu/Zn and Mn SOD but not with circulating cytokines (Table 1) in subjects with knee OA. In experimental mice, a decrease in Mn SOD impairs skeletal muscle function following collagen-induced arthritis [6] and Cu/Zn SOD deficiency associates with muscular weakness [13]. Conversely, muscular impairments induced by superoxide and other reactive oxygen or nitrogen species are protected by SOD in isolated skeletal muscle fibers from experimental mice [14]. Translating from pre-clinical findings, it is plausible that decreases in Cu/Zn and Mn SOD exacerbate muscular weakness, which subsequently, reduces the scavenging potential for superoxide and predisposes patients to a faster rate of disease progression. Although deviations in circulating SOD could represent the proteolytic cleavage from and reflect perturbations observed in arthritic chondrocytes during disease development and progression, further studies illuminating the source(s) of circulating SOD and the association of SOD with muscular-based and patient-reported outcomes in knee OA is desirable, especially when considering previous data demonstrating that a decrease in knee synovial fluid SOD associates with increasing pain [15].

Pain and physical dysfunction are clinical features of OA, and arguably, are the most important to patients and in determining if a TKA is necessary. In knee OA, IL-6 and IL-10 associate with pain and physical dysfunction [16]. Furthermore, longitudinal worsening of knee

Table 1
Correlation coefficients with the INV and NI legs muscular-based outcomes.

	NI					INV				
	pIsom			pIsok torque		pIsom			pIsok torque	
	force		torque	ext	flex	force		torque	ext	flex
	(N)	(N/kg)	(Nm)	(Nm)	(Nm)	(N)	(N/kg)	(Nm)	(Nm)	(Nm)
Cu/Zn SOD (ng/mL)	0.32	0.24	0.36	0.29	0.36	0.33	0.20	0.38*	0.32	0.39*
Mn SOD (ng/mL)	0.39*	0.30	0.38*	0.38*	0.41*	0.42*	0.31	0.43*	0.42*	0.47*
IL-1β (pg/mL)	0.11	0.11	0.10	0.07	−0.06	0.12	0.14	0.12	0.00	0.11
IL-2 (pg/mL)	0.10	0.10	0.01	0.04	−0.07	0.07	0.08	0.03	−0.08	0.08
IL-4 (pg/mL)	−0.10	−0.08	−0.08	−0.07	−0.14	−0.06	−0.03	−0.06	−0.12	−0.02
IL-5 (pg/mL)	−0.10	−0.01	−0.02	−0.04	−0.11	−0.03	0.08	0.03	−0.05	−0.02
IL-6 (pg/mL)	0.09	−0.21	0.02	−0.09	−0.21	0.06	−0.22	−0.05	0.03	−0.12
IL-7 (pg/mL)	−0.21	−0.11	0.00	−0.07	−0.09	−0.20	−0.13	0.04	−0.06	−0.06
IL-8 (pg/mL)	−0.25	−0.23	−0.01	−0.08	−0.19	−0.28	−0.27	0.02	−0.17	−0.13
IL-10 (pg/mL)	−0.20	−0.28	−0.19	−0.05	−0.10	−0.03	−0.12	−0.06	−0.04	−0.03
IL-12 (pg/mL)	0.12	0.34	0.16	0.31	0.23	0.12	0.34	0.13	0.17	0.25
IL-13 (pg/mL)	−0.09	0.04	−0.03	−0.06	−0.09	−0.01	0.14	0.00	−0.09	0.01
GM-CSF (pg/mL)	0.01	0.02	−0.10	−0.06	−0.09	−0.01	0.00	−0.10	−0.10	0.05
IFN-γ (pg/mL)	−0.03	0.01	−0.05	−0.01	−0.08	0.02	0.07	−0.04	−0.08	−0.01
TNF-α (pg/mL)	−0.04	−0.18	0.02	−0.01	−0.09	−0.06	−0.22	−0.06	0.00	0.01
sIL-1r1 (pg/mL)	−0.11	−0.09	−0.22	−0.21	−0.16	−0.13	−0.09	−0.29	−0.28	−0.11
sIL-1r2 (pg/mL)	0.11	0.02	0.14	0.08	0.01	−0.01	−0.08	0.04	−0.03	0.09
sIL-4r (pg/mL)	−0.23	0.09	−0.28	−0.18	−0.18	−0.18	0.13	−0.28	−0.32	−0.25
sIL-6r (pg/mL)	−0.01	0.01	0.06	0.00	0.08	−0.16	−0.16	−0.10	−0.18	0.04
sTNFr-1 (pg/mL)	−0.05	0.08	−0.03	0.01	0.05	−0.06	0.09	−0.09	−0.09	0.03
sTNFr-2 (pg/mL)	0.19	−0.02	0.07	0.13	0.15	0.18	0.03	0.11	0.16	0.15

pIsom, peak isometric; pIsok, peak isokinetic; ext, concentric knee extension; flex, concentric knee flexion.
* $p < 0.05$; n = 29.

Table 2
Correlation coefficients with patient-reported pain and dysfunction.

	Pain	Dysfunction
Cu/Zn SOD (ng/mL)	0.22	0.13
Mn SOD (ng/mL)	0.15	0.10
IL-1β (pg/mL)	−0.34	−0.38*
IL-2 (pg/mL)	−0.27	−0.36
IL-4 (pg/mL)	−0.39*	−0.38*
IL-5 (pg/mL)	−0.49*	−0.45*
IL-6 (pg/mL)	−0.01	0.01
IL-7 (pg/mL)	−0.26	−0.24
IL-8 (pg/mL)	−0.16	−0.26
IL-10 (pg/mL)	0.19	0.16
IL-12 (pg/mL)	−0.37*	−0.54*
IL-13 (pg/mL)	−0.44*	−0.48*
GM-CSF (pg/mL)	−0.28	−0.22
IFN-γ (pg/mL)	−0.45*	−0.44*
TNF-α (pg/mL)	−0.18	−0.10
sIL-1r1 (pg/mL)	−0.15	−0.12
sIL-1r2 (pg/mL)	0.17	0.00
sIL-4r (pg/mL)	−0.20	−0.55*
sIL-6r (pg/mL)	−0.02	−0.02
sTNFr-1 (pg/mL)	−0.17	−0.28
sTNFr-2 (pg/mL)	−0.13	0.02

* $p < 0.05$; $n = 29$.

pain associates with an elevation in baseline serum IL-6 [17], while an IL-6 gene polymorphism associates with an increase in serum IL-6 concentrations and patient-reported dysfunction in knee OA [18]. In this cross-sectional communication performed in subjects with knee OA, we extend those findings by providing unique data revealing an inverse association of pain or physical dysfunction with several cytokines (i.e., IL-1β, IL-4, IL-5, IL-12, IL-13, and/or interferon (IFN)-γ) but not with Cu/Zn or Mn SOD (Table 2). Based on data illustrating that synovial tissue features of inflammation [19] and both local and systemic cytokine concentrations decrease with disease severity [9,20], we speculate that the inverse association between patient-reported outcomes and circulating cytokines reflects an increase in localized pain and dysfunction and a decrease in the local cellular sources of cytokine production as a result of progressive joint tissue deterioration or loss during disease progression. Clearly, future research consisting of a comprehensive analysis of diverse cytokines addressing this premise is needed for later resolve as conflicting results indicate an alteration in pain with and without concurrent TNF-α and/or IL-6 perturbations [21,22].

In addition to various pro- and anti-inflammatory cytokines, this study provides new data suggesting that physical dysfunction inversely associates with the soluble IL-4 receptor (sIL-4r). The sIL-4r serves as a transport protein that protects and stabilizes IL-4 [23] and enhances IL-4 activity *in vivo* [24]. However, the ability of the sIL-4r to moderate the cellular responses mediated by IL-4 could be concentration dependent as low concentrations enhance and high concentrations neutralize the immune-modulatory properties regulated by IL-4 *in vitro* [23]. Therefore, the increase in circulating sIL-4r concentrations with knee OA [25] could marginalize the anti-inflammatory properties of IL-4 that protect against cartilage degradation in OA. Regarding patient-reported outcomes, however, results from this investigation suggest that increases in circulating IL-4 and the sIL-4r associate with a decrease in perceived physical dysfunction in subjects with knee OA (*see* Table 2). Although resolution of the enigma surrounding the role of IL-4 and the sIL-4r on clinical outcomes and disease progression is beyond the scope of the present report, the findings reported herein underscore the importance of additional research elucidating the sIL-4r role on patient-reported outcomes during OA progression, especially in the presence of concomitant IL-4 deviations.

There are a couple of study limitations worthy of discussion. First, this report consists of 29 subjects (16 females and 13 males), and second, only Cu/Zn and Mn SOD were examined of the endogenous antioxidant enzyme network. However, strict inclusion and exclusion criteria were implemented in an attempt to minimize the potential confounding influence of several variables (i.e., other diseases, current medication or treatment use, aging, physical activity level, and obesity) and SOD is a major scavenger of superoxide. While additional research is encouraged to include larger sample sizes of both genders and with a more robust analysis of endogenous antioxidant enzymes, this communication reveals unique relationships between contrasting systemic biomarkers and clinical outcomes in knee OA.

4. Conclusion

Inclusion of systemic biomarkers in the clinical evaluation of knee OA could complement standard of care procedures and guide the physician decision process by improving diagnostic and prognostic potential. Here, we provide original data correlating muscular-based outcomes to circulating SOD, while patient-reported outcomes inversely associate with circulating cytokines in subjects with knee OA. Based on these findings, we conclude that common but disparate outcome measures used in the assessment of OA progression associate with different systemic biomarkers. We speculate that low serum SOD in the presence of muscular weakness could identify a sub-group of patients vulnerable to a faster rate of disease progression, while low circulating cytokines with pain and dysfunction could represent imminent end-stage knee OA and identify candidates for TKA in the near future. Furthermore, these findings highlight the necessity of considering the potential rate of disease progression and disease severity when associating select biomarkers in the blood to OA outcomes in cross-sectional studies. Future longitudinal research identifying and comparing the temporal kinetics in circulating superoxide dismutases and cytokines during knee OA development and progression could reveal stage-specific therapeutic targets intended to improve patient care and outcomes.

Acknowledgements

We would like to thank all of the subjects that participated in this study. This study was funded in part by USANA Health Sciences, Inc. (Salt Lake City, UT USA).

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