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Secretory sphingomyelinase (S-SMase) activity is elevated in patients with rheumatoid arthritis

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Abstract

Objective—The goals of this study were to determine if secretory sphingomyelinase (S-SMase) activity is elevated in patients with rheumatoid arthritis (RA) compared to control subjects, and to examine the relationships of S-SMase activity with functional status, quality of life and RA disease activity measurements.

Methods—We collected data on 33 patients who were diagnosed with RA and 17 non- RA controls who were comparable in terms of age, sex and race. Demographic, clinical data and self-reported measures of fatigue, pain and physical function were obtained directly from patients and controls. RA patients also completed quantitative joint assessment using a 28-joint count and functional status and quality of life assessment using the Modified Health Assessment Questionnaire (mHAQ). Archived serum samples were used to analyze retrospectively serum S-SMase activity in patients and controls.

Results—The mean serum S-SMase activity was 1.4-fold higher in patients with RA (RA: 2.8 ± 1.0 nmol/mL/hr vs. Controls: 2.0 ± 0.8 nmol/mL/hr; $p=0.014$). Spearman's rho correlations between S-SMase activity and oxidant activity, markers of inflammation and endothelial activation with the exception of P-selectin ($\rho=0.40$, $p=0.034$), measures of disease activity, functional status and quality of life were not statistically significant in patients with RA.

Conclusion—We confirmed S-SMase activity is higher among RA patients compared to controls, as in other acute and chronic inflammatory diseases. Future studies can build on the present findings to understand more fully the biologic role(s) of S-SMase activity in RA.

Keywords

rheumatoid arthritis; biomarkers; cytokines; inflammation

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease characterized by inflammation of the synovium, which can lead to destruction of articular cartilage and juxtaarticular bone [1]. Accumulating evidence suggests a role for bioactive sphingolipid metabolites, such as ceramide, in various aspects of RA biology, including TNF α signaling

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and chemokine production [2]. Sphingomyelinases (SMases) are a family of enzymes that hydrolyze sphingomyelin to ceramide. Five different sphingomyelinases have been characterized until now, of which only one, the secretory sphingomyelinase (S-SMase), is found extracellularly. S-SMase is the Zn²⁺-dependent form of the acid sphingomyelinase, and it is released by many different human and murine cell types, including macrophages, fibroblasts, monocytes, endothelial cells and platelets [3,4].

Activation of SMases is the main metabolic pathway for the regulated production of ceramide during inflammation [5]. Elevated S-SMase activity in serum has been implicated with acute and chronic inflammatory conditions, such as cardiovascular disease, type 2 diabetes, chronic heart failure. In cardiovascular diseases, where the pathogenic role of S-SMase has been best characterized so far, S-SMase has been found to contribute to atherogenesis by promoting aggregation and subendothelial retention of aggregated low-density proteins (LDL) [6]. S-SMase activity and ceramide have also been associated with inflammation-induced muscle weakness. Clinical data show that sphingomyelinase activity correlates with elevated TNF levels and reduced skeletal muscle strength in patients with congestive heart failure, supporting a role for ceramide signaling in inflammation-induced muscle weakness [7]. Ceramide-induced weakness is mediated by a rise in muscle-derived oxidant activity, which depresses contractile function of skeletal muscle [8]. Elevated serum S-SMase activity has also been reported in humans and mice undergoing radiation treatment [9], or administered with LPS [10]. Pathogens like *S. typhi* and *E. coli*, as well as pro-inflammatory cytokines like TNF α and IL-1 β , acutely induce S-SMase release in macrophages, suggesting that S-SMase upregulation by inflammatory mediators may involve mechanisms shared by the innate immune response [11].

Although there is a growing understanding of the importance of sphingolipid signaling and an appreciation for their potential relevance in pathologic setting, peripheral blood levels of S-SMase activity and its biological role(s) in patients with RA has not been previously reported. Thus, the goals of this study were to determine if S-SMase activity is elevated in patients with RA compared to non-RA controls, and to examine the relationships of S-SMase activity with markers of inflammation, oxidant activity, RA disease activity, functional status and quality of life. Existing evidence implicates S-SMase activity in acute and chronic inflammatory diseases, cardiovascular disease and muscle weakness. Thus, we hypothesize that elevated S-SMase activity is associated with higher disease activity, more functional impairment and lower quality of life among patients with RA.

Patients and Methods

Study population

Previously collected subject data and archived sera from 33 patients with RA and 17 matched control subjects were used for this study. Patients with RA and control subjects participated in studies of inflammation and cardiovascular disease at Vanderbilt University Medical Center. Patients with RA met previously described inclusion and exclusion criteria and were studied between 2009 and 2011 as part of the baseline visit of a study of the anti-inflammatory effects of pioglitazone [12]. The control subjects were studied between 2013 and 2014 as part of a cardiac magnetic resonance imaging study, unpublished. Inclusion

criteria for the control subjects included age 18 years or older and lack of inflammatory or cardiac disease. The studies were approved by the Vanderbilt University Institutional Review Board, and all participants gave their written informed consent.

Demographic and clinical data (including medical history, physical exam, laboratory results and medications) were obtained directly from patients and controls. Self-reported measures of functional status and quality of life were obtained using the Modified Health Assessment Questionnaire (mHAQ). Self-reported measures of disease activity, fatigue and pain were assessed using visual analogue scales (VAS), ranging from 1–100 mm. Participants were also asked to quantify in minutes how long he/ she is limber as he/ she would be for the day and far in miles can he/ she walk comfortably. The 28-joint count and 8-iso PGF_{2a} (F₂-isoprostane) levels were assessed in RA patients, as measures of disease activity and oxidative stress, respectively. High sensitivity C-reactive protein (CRP) was measured in the hospital clinical laboratory. Archived serum samples were used to analyze S-SMase activity retrospectively.

In vitro Serum Sphingomyelinase Activity Assay

S-SMase activities were measured using C6-NBD-SM as a substrate [6]. Serum samples of patients and healthy controls were used as the source of S-SMase. The standardized assay contained serum (0.1 μ l), 20 μ M NBD-SM, 0.1 mM ZnCl₂, 0.1 M sodium acetate buffer (pH 5.0) in a final volume of 20 μ l. Assays containing 5 mM EDTA instead of Zn²⁺ were done in parallel and only the Zn²⁺-dependent increase was used to calculate the true S-SMase activity. Reactions were allowed to continue for 3 h at 37°C and were stopped by the addition of 0.5 ml of methanol. After further incubation at 37°C for 30 min, the samples were centrifuged at 1000 g and the generation of fluorescent product, NBD-ceramide, was monitored by reverse-phase HPLC using methanol/water/phosphoric acid (820:180:0.15, by vol.) as a mobile phase. The linearity of the assay in regards to sample volume and incubation time was confirmed using serial dilutions.

Statistical Analysis

Descriptive statistics are presented as frequencies and percentage (%) for categorical variables. Continuous variables were described with mean $\pm$ standard deviation. Comparisons of continuous variables between RA patients and non-RA controls were made using either a parametric T-test or a nonparametric rank-sum test, while comparisons of categorical variables between patients and controls were made using either a chi-square or Fisher's exact test. Spearman's correlations were used to examine the relationships of S-SMase activity and oxidant activity, measures of RA disease activity, inflammatory and endothelial activation markers, functional status and quality of life. Analyses were completed using IBM SPSS Statistics v24 software. Statistical significance was defined by a p-value < 0.05.

Results

RA patient and control subject characteristics are summarized in Table 1. Patients were on average 51 years old, and predominately female (82%) and non-Hispanic White (94%).

Among patients, 24% were current cigarette smokers, 36% were former smokers and 39% reported never smoking. Healthy controls were comparable to the patients in terms of age, sex, race and smoking status. In our cohort of RA patients, the mean DAS28 CRP was 4.6 ± 1.1 , consistent with moderate disease activity. Patients reported significantly longer periods of time before he/ she felt limber, reduced ability to walk comfortably and higher fatigue and pain levels compared with healthy controls ($p < 0.001$). In spite of this, the mean MHAQ score among patients was only 0.6 ± 0.5 , consistent with only mild, self-reported functional status limitation. About two thirds of patients were currently prescribed NSAIDs, and just over fifty percent were currently prescribed glucocorticosteroids. Approximately three quarters of patients were taking methotrexate, and less than one quarter of patients were on a biologic DMARD. The mean urinary F2-isoprostane level among patients was 2.3 ± 1.5 ng/mgCr.

Elevated S-SMase Activity in RA

The mean serum S-SMase activity was 1.4-fold higher in patients with RA (RA: 2.8 ± 1.0 nmol/mL/hr vs. Controls: 2.0 ± 0.8 nmol/mL/hr; $p = 0.014$) (Table 1, Figure 1).

Relationships between S-SMase Activity and F2- isoprostane, inflammatory and endothelial activation markers, measures of disease activity functional status and quality of life.

Spearman's rho correlations between S-Smase activity and F2-isoprostane ($\rho = 0.12$, $p = 0.52$), CRP ($\rho = 0.11$, $p = 0.55$), IL-6 ($\rho = 0.17$, $p = 0.39$), TNF α ($\rho = 0.24$, $p = 0.21$), neutrophil gelatinase-associated lipocalin-2 ($\rho = -0.045$, $p = 0.82$), sVCAM1 ($\rho = 0.111$, $p = 0.57$) and SAA ($\rho = 0.355$, $p = 0.06$) were not statistically significant. Spearman's rho correlation between S-Smase activity and P-selectin was significant ($\rho = 0.40$, $p = 0.034$). Spearman's rho correlations of serum S-Smase activity with other measures of disease activity including DAS28 CRP, functional status and quality of life in RA patients were also non-significant (Table 2).

Discussion

We confirmed S-SMase activity is higher among RA patients compared to controls, as in other acute and chronic inflammatory diseases. TNF, a key pro-inflammatory cytokine in RA, is known to stimulate sphingolipid metabolic enzymes such as sphingomyelinases [2]. Surprisingly, we did not find a significant relationship between serum TNF- α level and S-SMAase activity. One cause of the lack of correlation could be that other pro-inflammatory cytokines, i.e. IL-1 β that also stimulate S-SMase secretion may have additive effect to that of TNF α . Alternatively, cytokines produced locally that might also affect the S-SMase release, have remained unaccounted for. Among the biomarkers of inflammation and cardiovascular disease, soluble P-selectin was the only one to significantly correlated with S-SMase. Circulating soluble P-selectin is thought to trigger signaling in leucocytes that directly contributes to inflammation and thrombosis, and it is elevated in patients with cardiovascular diseases [12]. Soluble P-selectin has also been found to correlate with disease activity in RA patients with thrombocytosis [13]. In terms of the relationship between P-selectin and S-SMase, a recent study has implicated P-selectin in the regulation of S-SMase

release by platelets during tumor metastasis. Activation of wild type, but not P-selectin-deficient platelets by melanoma cells was shown to result in rapid and transient activation and secretion of S-SMase via mechanism involving p38 MAP kinase [14]. Additional studies are needed to establish if P-selectin regulates S-SMase activity in RA.

Previous studies have identified TNF-stimulated oxidant activity mediated by sphingolipid signaling as a cause for muscle dysfunction in chronic inflammatory disease, but we did not find an association between S-SMase level and measures of functional status and quality of life. This could be due to the selection of measures used to assess functional status in this study. One limitation of our study is that S-SMase activity was assessed retrospectively using archived serum samples. In conclusion, future studies can build on the present findings to understand more fully the relationships between S-SMase activity, inflammation, cardiovascular disease and skeletal muscle function in RA.

References

1. Harris ED Jr. Rheumatoid arthritis. Pathophysiology and implications for therapy *N Engl J Med*. 1990; 322(18):1277–1289. DOI: 10.1056/NEJM199005033221805 [PubMed: 2271017]
2. Maceyka M, Spiegel S. Sphingolipid metabolites in inflammatory disease *Nature*. 2014; 510(7503): 58–67. DOI: 10.1038/nature13475 [PubMed: 24899305]
3. Schissel SL, Schuchman EH, Williams KJ, Tabas I. Zn²⁺-stimulated sphingomyelinase is secreted by many cell types and is a product of the acid sphingomyelinase gene *J Biol Chem*. 1996; 271(31): 18431–18436. [PubMed: 8702487]
4. Jenkins RW, Canals D, Hannun YA. Roles and regulation of secretory and lysosomal acid sphingomyelinase *Cellular signalling*. 2009; 21(6):836–846. [PubMed: 19385042]
5. Marchesini N, Hannun YA. Acid and neutral sphingomyelinases: roles and mechanisms of regulation *Biochem Cell Biol*. 2004; 82(1):27–44. DOI: 10.1139/o03-091o03-091 [PubMed: 15052326]
6. Deevska GM, Sunkara M, Morris AJ, Nikolova-Karakashian MN. Characterization of secretory sphingomyelinase activity, lipoprotein sphingolipid content and LDL aggregation in *ldlr*^{−/−} mice fed on a high-fat diet *Bioscience reports*. 2012; 32(5):479–490. DOI: 10.1042/bsr20120036 [PubMed: 22712892]
7. Doehner W, Bunck AC, Rauchhaus M, von Haehling S, Brunkhorst FM, Ciccoira M, Tschope C, Ponikowski P, Claus RA, Anker SD. Secretory sphingomyelinase is upregulated in chronic heart failure: a second messenger system of immune activation relates to body composition, muscular functional capacity, and peripheral blood flow *Eur Heart J*. 2007; 28(7):821–828. DOI: 10.1093/eurheartj/ehl541 [PubMed: 17353227]
8. Reid MB, Moylan JS. Beyond atrophy: redox mechanisms of muscle dysfunction in chronic inflammatory disease *J Physiol*. 2011; 589(Pt 9):2171–2179. DOI: 10.1113/jphysiol.2010.203356 [PubMed: 21320886]
9. Sathishkumar S, Boyanovsky B, Karakashian AA, Rozenova K, Giltaiy NV, Kudrimoti M, Mohiuddin M, Ahmed MM, Nikolova-Karakashian M. Elevated sphingomyelinase activity and ceramide concentration in serum of patients undergoing high dose spatially fractionated radiation treatment: implications for endothelial apoptosis *Cancer Biol Ther*. 2005; 4(9):979–986. doi:1915 [pii]. [PubMed: 16096366]
10. Bourbon NA, Sandirasegarane L, Kester M. Ceramide-induced inhibition of Akt is mediated through protein kinase C ζ : implications for growth arrest *J Biol Chem*. 2002; 277(5):3286–3292. M110541200 [pii]. DOI: 10.1074/jbc.M110541200 [PubMed: 11723139]
11. McCollister BD, Myers JT, Jones-Carson J, Voelker DR, Vazquez-Torres A. Constitutive acid sphingomyelinase enhances early and late macrophage killing of *Salmonella enterica* serovar Typhimurium *Infection and immunity*. 2007; 75(11):5346–5352. DOI: 10.1128/iai.00689-07 [PubMed: 17698574]

12. Panicker SR, Mehta-D'souza P, Zhang N, Klopocki AG, Shao B, McEver RP. Circulating soluble P-selectin must dimerize to promote inflammation and coagulation in mice *Blood*. 2017; 130(2): 181–191. DOI: 10.1182/blood-2017-02-770479 [PubMed: 28515093]
13. Ertenli I, Kiraz S, Arici M, Haznedaroglu IC, Calguneri M, Celik I, Kirazli S. P-selectin as a circulating molecular marker in rheumatoid arthritis with thrombocytosis *J Rheumatol*. 1998; 25(6):1054–1058. [PubMed: 9632062]
14. Becker KA, Beckmann N, Adams C, Hessler G, Kramer M, Gulbins E, Carpinteiro A. Melanoma cell metastasis via P-selectin-mediated activation of acid sphingomyelinase in platelets *Clinical & experimental metastasis*. 2017; 34(1):25–35. DOI: 10.1007/s10585-016-9826-6 [PubMed: 27744579]

* $p=0.014$

Figure 1.
S-SMase activity in RA and non-RA controls

Table 1.

Characteristics of the 33 patients with RA and 17 non-RA controls

Characteristic	RA (n=33)	Non-RA control (n=17)
Current age, mean $\pm$ SD years	50.9 $\pm$ 14.4	50.8 $\pm$ 11.8
Sex, Female, count (%)	27 (82)	14 (82)
Race, count (%)		
American Indian or Alaskan Native	2 (6)	0
Black or African American	2 (6)	1 (6)
White	28 (85)	16 (94)
More than one race	1 (3)	0
Hispanic or Latino, count (%)	2 (6)	0
Smoking status, count (%)		
Current	8 (24)	2 (12)
Former	12 (36)	7 (41)
Never	13 (39)	8 (47)
RF, no. positive/ no. tested	21/26	N/A
CRP, mean $\pm$ SD mg/L	12.9 $\pm$ 25.3	4.7 $\pm$ 8.1
DAS28 CRP, mean $\pm$ SD units	4.6 $\pm$ 1.1	ND
MHAQ score, mean $\pm$ SD units	0.6 $\pm$ 0.5	ND
How long before he/she is as limber as he/ she would be for the day min, mean $\pm$ SD *	52.2 $\pm$ 52.6	2.4 $\pm$ 4.0
How far can he/ she walk comfortably miles, mean $\pm$ SD *	1.1 $\pm$ 1.4	3.4 $\pm$ 4.1
Fatigue 1–100mm VAS, mean $\pm$ SD *	43.2 $\pm$ 30.1	10.1 $\pm$ 21.1
Pain in the past week 1–100mm VAS, mean $\pm$ SD *	47.5 $\pm$ 4.0	4.7 $\pm$ 5.0
Self-reported disease activity 1–100mm VAS, mean $\pm$ SD *	39.2 $\pm$ 27.5	2.4 $\pm$ 2.3
Any NSAID in the past month	22 (67)	12 (70)
Any glucocorticosteroids in the past month	18 (54)	1 (6)
DMARD use, count (%)		
Methotrexate	24 (73)	ND
Leflunomide	5 (15)	ND
Sulfasalazine	1 (3)	ND
Hydroxychloroquine	6 (18)	ND
Azathioprine	0	ND
Etanercept	7 (21)	ND
alimumab	5 (15)	ND
Infliximab	2 (6)	ND
Rituximab	1 (3)	ND
Abatacept	2 (6)	ND
Sphingomyelinase, mean $\pm$ SD **	2.7 $\pm$ 1.0	2.0 $\pm$ 0.8
F2-isoprostane, mean $\pm$ SD ** ng/ mgCr	2.3 $\pm$ 1.5	ND

* $p < 0.0001$ ** $p = 0.014$

Table 2.

Spearman's rho correlations of S-Smase activity and F2-isoprostane, measures of disease activity, functional status and quality of life in RA patients

	S-Smase activity p (p)
How long before he/she is as limber as he/ she would be for the day min	-0.12 (0.495)
How far can he/ she walk comfortably miles	0.24 (0.191)
Fatigue 1–100mm VAS	-0.20 (0.263)
Pain in the past week 1–100mm VAS	-0.24 (0.178)
Self-reported disease activity 1–100mm VAS	-0.06 (0.771)
MHAQ score	-0.22 (0.225)
DAS28 CRP score	-0.21 (0.243)