Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 58 (2009) 316–318
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Plasma asymmetric dimethylarginine is related to anticitrullinated protein antibodies in rheumatoid arthritis of short duration

Andrzej Surdacki[a,*], Jens Martens-Lobenhoffer[b], Alicja Wloch[c], Piotr Gluszko[d], Tomasz Rakowski[a], Jacek S. Dubiel[a], Stefanie M. Bode-Böger[b]

[a]*2nd Department of Cardiology, Jagiellonian University, 31-501 Cracow, Poland*
[b]*Institute of Clinical Pharmacology, Otto-von-Guericke University, D-39120 Magdeburg, Germany*
[c]*Center for Rheumatology, Immunology and Rehabilitation, 30-119 Cracow, Poland*
[d]*Department of Rheumatology, Jagiellonian University, 31-531 Cracow, Poland*

Received 7 May 2008; accepted 30 October 2008

Abstract

We have recently demonstrated elevated plasma levels of an endogenous nitric oxide synthase inhibitor, asymmetric dimethyl-L-arginine (ADMA), and its association with carotid atherosclerosis in rheumatoid arthritis (RA). Both an elevated risk of myocardial infarction and increased levels of anticitrullinated protein antibodies (ACPAs), specific for RA, had been shown to precede the onset of clinical RA symptoms. Therefore, our aim was to verify the hypothesis that ADMA accumulation might accompany raised ACPAs titers in RA of short duration (≤3 years). Twenty patients (16 women, 4 men; mean age, 45 ± 12 years; mean disease duration, 2.3 ± 0.5 years) with active RA despite chronic disease-modifying antirheumatic medication, free of cardiovascular disease or atherosclerotic risk factors, were studied. Plasma levels of ADMA and its stereoisomer, symmetric dimethyl-L-arginine (SDMA), were assayed by liquid chromatography/tandem mass spectrometry. The ACPAs were measured by a second-generation enzyme-linked immunosorbent assay. In addition to routine biochemical assays, plasma concentrations of tumor necrosis factor α, monocyte chemoattractant protein–1, and vascular cell adhesion molecule–1 soluble form were analyzed with respective enzyme-linked immunosorbent assays. A significant positive correlation between levels of ACPAs and ADMA ($r = .60$, $P = .005$), but not SDMA ($r = -.02$, $P = .9$), was found. Neither ADMA nor SDMA was correlated to any of the clinical or biochemical parameters reflecting disease activity and inflammatory activation. Thus, excessive ADMA accumulation accompanies elevated ACPAs levels in patients with RA of short duration free of cardiovascular disease or risk factors.

We have recently described elevated plasma levels of an endogenous nitric oxide formation inhibitor, asymmetric dimethyl-L-arginine (ADMA), and its association with carotid atherosclerosis in rheumatoid arthritis (RA) [1]. Accumulation of ADMA, an independent adverse outcome predictor in coronary artery disease, had previously been demonstrated in nonatherosclerotic patients with traditional risk factors, being related to the magnitude of endothelial dysfunction, an antecedent of atherosclerosis [2]. A 3- to 6-fold increase in the risk of myocardial infarction was reported already before the RA diagnosis [3]. Anticitrullinated protein antibodies (ACPAs), highly specific for RA, also precede the onset of RA clinical symptoms, with the prevalence of ACPAs positivity increasing over time and amounting to 52% within 18 months before fulfillment of RA diagnostic criteria [4]. The ACPAs positivity was recently linked to subclinical carotid atherosclerosis in RA patients with a mean disease duration of 11 years [5]. Our aim was to verify the hypothesis that ADMA accumulation might accompany raised ACPAs titers in RA of short duration.

Inclusion/exclusion criteria had been described in detail [1] and were supplemented with a disease duration not exceeding 3 years as a necessary rule-in condition. In brief, we studied 20 patients with active RA despite chronic disease-modifying antirheumatic drugs therapy (16 women, 4 men; age, 45 ± 12 years; disease duration, 2.3 ±

* Corresponding author. Fax: +48 12 636 8039.
E-mail address: surdacki.andreas@gmx.net (A. Surdacki).

0026-0495/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2008.10.002

0.5 years) free of cardiovascular disease, risk factors, or severe extraarticular RA manifestations.

Patients' characteristics included demographical data, Disease Activity Score in 28 joints, disease duration, rheumatoid factor positivity, body mass index, blood pressure, estimated glomerular filtration rate, fasting levels of glucose, homocysteine, triglycerides, as well as low-density and high-density lipoprotein cholesterol. Plasma ADMA and its stereoisomer, symmetric dimethyl-L-arginine (SDMA), were determined by liquid chromatography/tandem mass spectrometry [1]; enzyme-linked immunosorbent assays were used to measure ACPAs (Euroimmun AG, Luebeck, Germany), tumor necrosis factor α, monocyte chemoattractant protein–1, and vascular cell adhesion molecule–1 soluble form (R&D Systems, Minneapolis, MN), whereas chemiluminescence immunoassay was used for high-sensitivity C-reactive protein and homocysteine (DPC, Flanders, NJ). Spearman rank correlation coefficients (r) between ADMA or SDMA and the above-listed variables were computed.

Levels of ACPAs and ADMA, but not SDMA, were interrelated (Fig. 1). Neither ADMA nor SDMA correlated to any of the clinical or biochemical parameters ($P > .1$).

We had previously mentioned proinflammatory cytokines, oxidative stress, potentiated endothelial cell turnover, and hypoxia in the inflamed synovium as possible explanations of elevated ADMA with unchanged SDMA in RA [1]. However, the selective association between ADMA and ACPAs suggests that ACPAs might be closer to events responsible for ADMA accumulation.

Anticitrullinated protein antibodies are generated in response to the conversion of charged peptidylarginine residues to uncharged peptidylcitrulline by peptidylarginine deiminase (PAD) isotypes 2 and 4 in synovial monocytes/macrophages and lymphocytes [6,7]. The ACPAs levels correlated to the presence of RA-specific intracellular, but not extracellular, citrullinated proteins [8]. In addition, PAD-4 polymorphism was linked to RA [9] and higher ACPAs in RA of short duration [10].

The activity of PAD-4, the only nuclear PAD isotype [7,11], might interfere with the formation of ADMA, known to be generated mostly in nucleic acids–binding proteins [12]. In histones, the PAD-4–mediated deimination competed for the same peptidylarginine residues against asymmetric methylation via protein-arginine type I *N*-methyltransferases (PRMTs-I) [13,14], catalyzing ADMA (not SDMA) synthesis [2]. Nevertheless, even if these interactions had been sufficient to inhibit whole-body ADMA metabolism, their effect might have been an inverse ACPAs-ADMA relation, that is, opposite to our finding, insofar as ACPAs reflect intracellular proteins citrullination [8].

In systemic lupus erythematosus, the association of ADMA with cardiovascular events history and anti-DNA titers [15] was explained by the ability of anti-DNA, cross-reacting with the arginine-glycine–rich domain of heterogeneous nuclear ribonucleoprotein A2 (hnRNP A2), to stimulate PRMTs-I [16]. As arginine-glycine-glycine is a preferential sequence for ADMA formation [12] and as deimination and asymmetric methylation share common sites [13,14], citrulline-containing epitopes recognized by ACPAs hypothetically localize in arginine/glycine-rich regions. Whether this might possibly facilitate PRMTs-I–dependent ADMA formation—by analogy with the effect of a PRMT-I in hnRNP A2 preincubated with anti-DNA [16]—remains to be verified in an experimental study. In addition, even demonstration of such a phenomenon in hnRNP A2 in vitro would not allow its simple extrapolation into citrullinated proteins recognized by ACPAs in RA synovium.

The proposed mechanism, although speculative, may—if confirmed—constitute a novel proatherosclerotic pathway in RA.

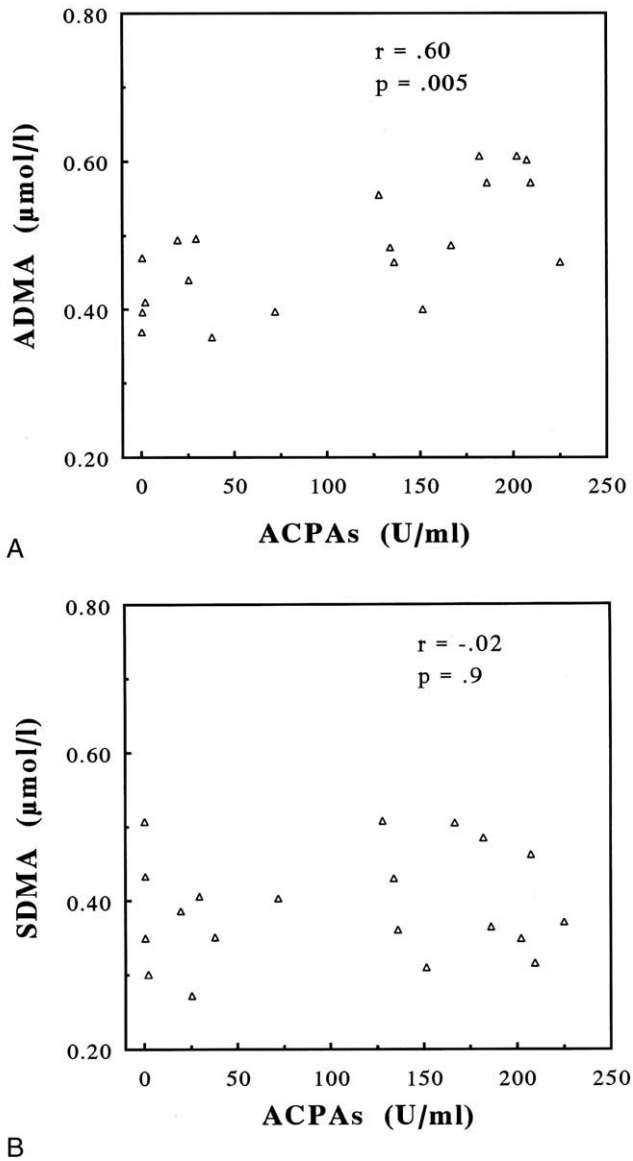

Fig. 1. A positive correlation between plasma ACPAs and ADMA (A), but not SDMA (B) levels.

References

[1] Surdacki A, Martens-Lobenhoffer J, Wloch A, Marewicz E, Rakowski T, Wieczorek-Surdacka E, et al. Elevated plasma asymmetric dimethyl-L-arginine levels are linked to endothelial progenitor cell depletion and carotid atherosclerosis in rheumatoid arthritis. Arthritis Rheum 2007;56:809-19.

[2] Vallance P, Leiper J. Cardiovascular biology of the asymmetric dimethylarginine: dimethylarginine dimethylaminohydrolase pathway. Arterioscler Thromb Vasc Biol 2004;24:1023-30.

[3] Maradit-Kremers H, Crowson CS, Nicola PJ, Ballman KV, Roger VL, Jacobsen SJ, et al. Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study. Arthritis Rheum 2005;52:402-11.

[4] Rantapää-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 2003;48:2741-9.

[5] Gerli R, Bartoloni Bocci E, Sherer Y, Vaudo G, Moscatelli S, Shoenfeld Y. Association of anti-cyclic citrullinated peptide antibodies with subclinical atherosclerosis in patients with rheumatoid arthritis. Ann Rheum Dis 2008;67:724-5.

[6] Chang X, Yamada R, Suzuki A, Sawada T, Yoshino S, Tokuhiro S, et al. Localization of peptidylarginine deiminase 4 (PADI4) and citrullinated protein in synovial tissue of rheumatoid arthritis. Rheumatology 2005;44:40-50.

[7] Foulquier C, Sebbag M, Clavel C, Chapuy-Regaud S, Al Badine R, Méchin MC, et al. Peptidyl arginine deiminase type 2 (PAD-2) and PAD-4 but not PAD-1, PAD-3, and PAD-6 are expressed in rheumatoid arthritis synovium in close association with tissue inflammation. Arthritis Rheum 2007;56:3541-53.

[8] De Rycke L, Nicholas AP, Cantaert T, Kruithof E, Echols JD, Vandekerckhove B, et al. Synovial intracellular citrullinated proteins colocalizing with peptidyl arginine deiminase as pathophysiologically relevant antigenic determinants of rheumatoid arthritis–specific humoral autoimmunity. Arthritis Rheum 2005; 52:2323-30.

[9] Suzuki A, Yamada R, Chang X, Tokuhiro S, Sawada T, Suzuki M, et al. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet 2003;34:395-402.

[10] Cha S, Choi CB, Han TU, Kang CP, Kang C, Bae SC. Association of anti-cyclic citrullinated peptide antibody levels with PADI4 haplotypes in early rheumatoid arthritis and with shared epitope alleles in very late rheumatoid arthritis. Arthritis Rheum 2007;56:1454-63.

[11] Nakashima K, Hagiwara T, Yamada M. Nuclear localization of peptidylarginine deiminase V and histone deimination in granulocytes. J Biol Chem 2002;277:49562-8.

[12] Gary JD, Clarke S. RNA and protein interactions modulated by protein arginine methylation. Prog Nucleic Acid Res Mol Biol 1998; 61:65-131.

[13] Wang Y, Wysocka J, Sayegh J, Lee YH, Perlin JR, Leonelli L, et al. Human PAD4 regulates histone arginine methylation levels via demethylimination. Science 2004;306:279-83.

[14] Cuthbert GL, Daujat S, Snowden AW, Erdjument-Bromage H, Hagiwara T, Yamada M, et al. Histone deimination antagonizes arginine methylation. Cell 2004;118:545-53.

[15] Bultink IE, Teerlink T, Heijst JA, Dijkmans BA, Voskuyl AE. Raised plasma levels of asymmetric dimethylarginine are associated with cardiovascular events, disease activity, and organ damage in patients with systemic lupus erythematosus. Ann Rheum Dis 2005; 64:1362-5.

[16] Sun KH, Tang SJ, Wang YS, Lin WJ, You RI. Autoantibodies to dsDNA cross-react with the arginine-glycine-rich domain of heterogeneous nuclear ribonucleoprotein A2 (hnRNP A2) and promote methylation of hnRNP A2. Rheumatology 2003;42:154-61.