Evaluation of Paraoxonase Activity in Patients with Mixed Connective Tissue Disease

EDIT BODOLAY, ILDIKO SERES, PETER SZODORAY, ISTVAN CSÍPO, ZSANETT JAKAB, JUDIT VEGH, ANNA SZILAGYI, GYULA SZEGEDI, and GYORGY PARAGH

ABSTRACT. ***Objective.*** Mixed connective tissue disease (MCTD) is a systemic inflammatory autoimmune disease. The connection between inflammatory measures and atherosclerosis in MCTD has not been described. Paraoxonase (PON) is known to have an antioxidant function. We evaluated lipid profiles and PON activity in patients with MCTD.

Methods. Thirty-seven patients with MCTD were enrolled. Patients had taken no antihyperlipidemic drugs in the past 2 months. Thirty healthy individuals served as controls. The mean age of patients was 51.2 ± 9.5 years; disease duration was 11.0 ± 7.2 years. PON activity was determined with spectrophotometry, von Willebrand factor (vWF) antigen was investigated with the immunoturbidimetry method, and thrombomodulin and antiendothelial cell antibody (AECA) measurements were carried out by ELISA.

Results. PON activity in patients with MCTD was significantly lower than in the controls (patients 118.5 ± 64.6 U/l, controls 188.0 ± 77.6; $p < 0.001$). Arylesterase activity was significantly reduced in patients ($p < 0.001$). Reduction of PON activity showed a close correlation with the age of the patients, duration of the disease, and vascular events (eye, cardiac, cerebral). There was a close association between the low PON activity and endothelial cell activation markers (thrombomodulin, vWF, AECA).

Conclusion. Our results indicate that in patients with MCTD there is an increased risk for atherosclerosis. In the development of atherosclerosis, besides the elevated levels of cholesterol and triglyceride, reduced PON concentrations and PON activity may play a crucial role. (First Release Dec 15 2007; J Rheumatol 2008;35:237–43)

Key Indexing Terms:
MIXED CONNECTIVE TISSUE DISEASE
PARAOXONASE
LIPID LEVELS
VASCULAR DAMAGE

Currently, prominent areas of study in the systemic autoimmune diseases [e.g., systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and systemic vasculitis] are atherosclerosis and protection against atherosclerosis[1-4]. Cardiovascular mortality in SLE is 30% of the total mortality in patients with SLE[5,6]. Recent studies unequivocally verified that the causes of vascular damage in systemic autoimmune diseases are the same as the common known pathophysiological factors of atherosclerosis; moreover, patients with systemic autoimmune diseases have high levels of circulating proinflammatory cytokines, namely tumor necrosis factor-α and interleukin 1[7]. Local expression of endothelial cell-surface adhesion molecules facilitates migration of granulocytes and monocytes from the circulation to the tissues; further, oxidatively modified low density lipoprotein (LDL) — acting through the scavenger receptor of macrophages — enhances the production of foamy cells, which lead to early and rapid development of atherosclerosis[8].

In addition to inflammation, one of the most important risk factors for atherosclerosis developing in young individuals with systemic autoimmune disease is dyslipoproteinemia. High-density lipoprotein (HDL) has numerous roles as a protective agent against atherosclerosis; one is that it provides reverse cholesterol transport, as it carries away excessive cholesterol from the tissues, while its other role is the antiatherogenic function. HDL binds paraoxonase (PON) enzyme produced by the liver through Apo1 and inhibits LDL oxidation[9,10].

Mixed connective tissue disease (MCTD) is a systemic inflammatory autoimmune disease[11-14]; typical symptoms are Raynaud's phenomenon, polyarthirits, swollen hands and fingers, myositis, esophageal hypomotility, and the cutaneous symptoms such as erythema, telangiectasia, hypo/hyperpigmentation and sclerodactyly. Renal involvement is rarer than

From the Division of Clinical Immunology, 3rd Department of Internal Medicine, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary.

E. Bodolay, MD, PhD; P. Szodoray, MD, PhD; I. Csipo, PhD; J. Vegh, MD, PhD; A. Szilagyi, Division of Clinical Immunlogy, 3rd Department of Internal Medicine, Medical and Health Science Center, University of Debrecen; I. Seres, MD, PhD; Z. Jakab; G. Paragh, MD, PhD, 1st Department of Internal Medicine, Medical and Health Science Center, University of Debrecen; G. Szegedi, MD, PhD, Research Group of Autoimmune Diseases, Hungarian Academy of Sciences, Coordination Institute of Clinical Immunology and Allergology, Debrecen.

Address reprint requests to Dr. E. Bodolay, Division of Clinical Immunlogy, 3rd Department of Internal Medicine, Medical and Health Science Center, University of Debrecen, Moricz Zs. str 22, 4032 Debrecen, Hungary. E-mail: bodolai@iiibel.dote.hu

Accepted for publication September 28, 2007.

in SLE, observed in 5%–10% of cases. Sera of patients with MCTD have specific autoantibodies to small-nuclear U1RNP. Patients with MCTD also have risk factors for early development of atherosclerosis. Levels of proinflammatory cytokines are continuously elevated in the patients' sera[15-17]. Proliferating vascular arteriopathy is a specific feature of MCTD.

The histological characteristics of this abnormality are intima thickening of the arteriolae, media hypertrophy, arteritis, and development of pexiform lesions. Endothelial cells are activated and expression of adhesion proteins further increases the inflammation of the tissue[18]. Lipoprotein abnormalities have been described in a few individuals with MCTD, but no evaluation data derived from a larger group of patients has been reported[19,20].

Our aim was to evaluate serum lipid profile in patients with MCTD. We assessed the level and activity of the main natural antioxidant, PON, in the sera of patients with MCTD. We determined changes in PON activity in correlation with vascular events in patients with MCTD, and also assessed serum concentrations of the endothelial cell activation markers thrombomodulin (TM) and von Willebrand factor (vWF) antigen.

MATERIALS AND METHODS

We enrolled 37 patients (36 women, 1 man) with MCTD for study; all were treated and followed at the outpatient clinic for autoimmune patients of the 3rd Department of Internal Medicine, Medical and Health Science Center, University of Debrecen, between November 2003 and March 2004. MCTD was diagnosed based on the Alarcon-Segovia and Villareal criteria[21]; all 37 patients fulfilled the criteria. Smokers were excluded due to the PON activity measurements; no patient received lipid-lowering drugs in a 2-month period before the study. The results were compared to the data of 30 healthy individuals (27 women, 3 men) of corresponding sex and age who served as controls. Thirteen patients out of 37 with MCTD received nonsteroidal anti-inflammatory drugs, 8 patients had the combination of corticosteroids and salazopyrin, 11 patients received combination therapy of corticosteroids and methotrexate, while 5 patients recived corticosteroid therapy. The mean dosage of corticosteroid was 8.2 mg/day.

Patients with MCTD were followed up every 4 months. At each visit we performed chest radiographs, respiratory function tests, and Doppler echocardiography; high-resolution computed tomography imaging of patients was performed once a year. In addition to recording physical status, we assessed erythrocyte sedimentation rate, renal and liver function tests and urinalysis were performed, and C-reactive protein (CRP) levels were determined.

Clinical variables. Ophthalmologic abnormality was diagnosed if visual disturbance was caused by retinal abnormality or optic nerve atrophy. Cerebrovascular diseases were diagnosed if the neurological abnormality detected by the neurologist was also verified by either CT or magnetic resonance imaging (MRI). Coronary disease was diagnosed if (1) the patient had an acute myocardial infarction or had the signs of myocardial infarctions on the ECG performed semiannually; (2) the disease was treated by means of coronary bypass surgery or angioplasty; or (3) angina was verified by angiography and/or the ischemic alterations were verified by a noninvasive test or tissue Doppler examination.

Serum lipid measurements. Determination of serum lipid measures was performed at the Department of Clinical Biochemistry and Molecular Pathology, University of Debrecen. Lipoprotein(a), triglyceride, and HDL-C were determined by turbidimetry, using a Roche Integra 700 analyzer (Roche Diagnostics, Basel, Switzerland). Serum lipoprotein electrophoresis was performed on agarose gel and measurements were completed using a Cliniscan densitometer (Helena, Beaumont, TX, USA). We defined hypercholesterolemia as serum cholesterol concentrations > 5.2 mmol/l, as determined by photometry based on a peroxidase reaction. We used Friedewald's formula to calculate LDL concentrations, while determination of concentrations of apolipoprotein A1 and B was performed with an Orion Diagnostica kit (Orion Diagnostica, Espoo, Finland), according to the manufacturer's instructions. The system employs an immune-nephelometric method.

Measuring PON activity. We used paraoxon (O,O-diethyl-O-p-nitrophenylphosphate; Sigma, St. Louis, MO, USA) as a substrate during measurements of serum PON activity, which was transformed to 4-nitrophenol as an effect of the PON enzyme present in the serum, leading to an increase of absorption measured at 412 nm. During the measurements, we added 1 ml Tris/HCl buffer (100 mM, pH 8.0), which contained 2 mmol $CaCl_2$ and 5.5 mmol/l paraoxon to 50 μl of patient serum. Production of 4-nitrophenol was monitored at 412 nm at 25°C with a Hewlett-Packard 8453 UV-Visible spectrophotometer. Enzyme activity was calculated using the molar extinction coefficient (17,100 $M^{-1}cm^{-1}$)[22].

Determining phenotype distribution of PON. The determination of phenotype distribution of PON was completed with the double substrate method, using paraoxon as one substrate and phenylacetate as the other. Using paraoxon as substrate, we added NaCl in a concentration of 1 M in order to measure the NaCl-stimulated activity. The conformation of the active binding site of the enzyme changes in the presence of NaCl, which facilitates the binding and faster transformation of paraoxon, leading to an increased detectable PON activity. The phenylacetate substrate was used to measure the arylesterase activity of the enzyme. In these measurements we added 1 mM phenylacetate solution in 20 mM Tris/HCl buffer (pH 8.0) to the sera and monitored the absorption increase at 270 nm. The ratio of NaCl-stimulated PON activity and the arylesterase activity identifies the 3 distinct phenotypes characterized by different levels of activity: AA (low activity, homozygous), AB (intermediate activity, heterozygous), and BB (high activity, homozygous).

Immunoserological investigations

Autoantibodies. The autoantibodies (anti-U1RNP, anti-ENA, anti-Sm, anti-SSA, anti-SSB, anti-Jo1, anti-Scl70, anti-DNA, and anticardiolipin antibodies) were detected by ELISA method (Cogent Diagnostics, Edinburgh, UK and Pharmacia; Upjohn Diagnostic GmbH, Freiburg, Germany).

vWF antigen. Level of vWF antigen was determined using platelet-poor plasma and assessed by an immunoturbidimetric method using a STA-Liatest vWF kit (Diagnostica Stago, Asnieres, France) on a Stago Star Compact analyzer (Roche Diagnostics).

Serum thrombomodulin (TM). Determination of TM level was performed by enzyme immunoassay (Diagnostica Stago). The wells of the ELISA plate were covered with monoclonal antithrombomodulin antibody. We added standard and control samples to the wells and the citrate plasma derived from patients and healthy individuals, diluted 1:5. The plate was incubated 2 h at room temperature and then rinsed 5 times using 200 μl rinsing buffer. Then we added 200 μl of immunoconjugate to the wells, followed by 2-h incubation at room temperature. After rinsing the plate we added 200 μl of OPD/urea peroxide substrate to wells. The reaction was stopped by adding 50 μl of 3 M sulfuric acid to the wells. Absorption measurements were performed on a Multiscan MS ELISA reader (Labsystems, Helsinki, Finland) at 492 nm. A calibration curve was obtained using the data derived from the absorption measurements and the corresponding known concentration values. TM concentrations were read off the curve in ng/ml.

Antiendothelial cell antibodies (AECA). Examination of AECA was performed on endothelial cells from human umbilical cord veins, employing an ELISA method as described[18].

Statistical analyses. Statistical analysis of measurement data was performed with SPSS 13.0 software. We utilized a 2-sample Student t-test and nonparametric Mann-Whitney test, Spearman correlation analysis, and chi-square test. Non-normally distributed data were transformed logarithmically to correct their skewed distributions. Values are given as mean ± deviation. Results were considered significant at $p < 0.05$.

RESULTS

The demographic data and clinical features of patients with MCTD are summarized in Table 1. The mean age of patients with MCTD at the time of the survey was 51.2 ± 9.5 years. The mean followup period was 11.0 ± 7.2 years. No significant difference in body mass index (BMI) was observed between the patients and controls (BMI for patients 25.27 ± 3.22 kg/m^2; for controls 23.62 ± 2.92 kg/m^2; nonsignificant). Patients with MCTD had significantly higher serum levels of total cholesterol and triglyceride compared to the control group for corresponding ages [total cholesterol in patients 6.3 ± 1.4 mmol/l; in controls 5.17 ± 0.8 mmol/l ($p < 0.001$); triglyceride in patients 1.5 ± 0.8 mmol/l, in controls 1.08 ± 0.48 mmol/l ($p < 0.01$; Figure 1)]. No difference was found in HDL and LDL cholesterol concentrations of patients compared to controls.

The quantity of ApoA1 was significantly lower in patients than in controls, while there was no difference in the quantity of ApoB apoprotein between patients and controls [ApoA1 in patients 1.6 ± 0.35 g/l, in controls 1.87 ± 0.28 g/l ($p < 0.01$); ApoB in patients 0.97 ± 0.36 g/l, in controls 0.98 ± 0.7 g/l (nonsignificant; Figure 2)].

The quantity of PON was decreased in the sera of patients with MCTD compared to the control group; further,

Table 1. Demographic data and clinical symptoms of patients with MCTD.

Feature	Patients, n = 37 (%)	Controls, n = 30 (%)
Female/male	36/1	27/3
Age at the time of the survey, yrs ± SD	51.2 ± 9.5	46.57 ± 9.9
Duration of MCTD, yrs ± SD	11.0 ± 3.22	NA
BMI, kg/m^2	25.27 ± 3.22	23.62 ± 2.92
Clinical features		
Polyarthritis	32 (86)	NA
Raynaud's phenomenon	29 (78)	NA
Esophageal motility dysfunction	20 (54)	NA
Myositis	21 (56)	NA
Abnormalities of respiratory organs (pulmonary arterial hypertension, interstitial pulmonary disease)	19 (51)	NA
Skin symptoms (photosensitivity, hypo- and hyperpigmentation, telangiectasia)	12 (32)	NA
Cerebrovascular abnormalities	11 (30)	NA
Cardiovascular abnormalities (myocardial infarction, angina pectoris, ischemic heart disease)	21 (56)	NA
Circulatory disturbances of retina	9 (24)	NA

NA: not applicable.

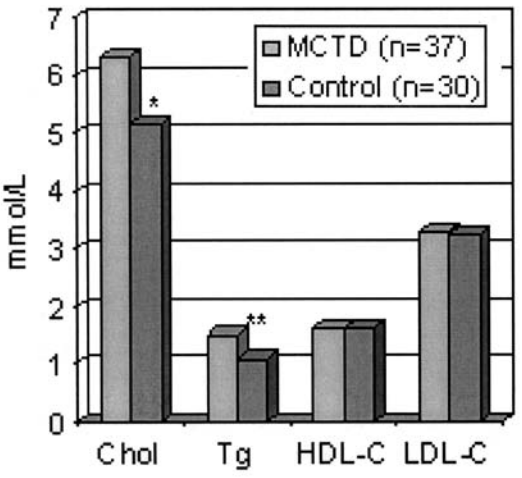

Figure 1. Lipoprotein concentrations in sera of patients with MCTD. Patients with MCTD had significantly higher serum levels of total cholesterol and triglyceride compared to controls. No difference was found in HDL and LDL cholesterol levels of patients with MCTD and controls. *$p < 0.001$; **$p < 0.01$. Chol: total cholesterol, Tg: triglycerides, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol.

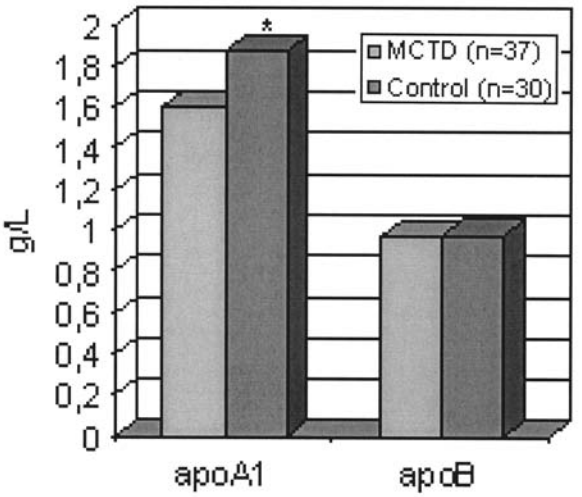

Figure 2. Levels of ApoA1 and ApoB in sera of patients with MCTD. The quantity of ApoA1 was significantly lower in patients than in controls, while there was no difference in quantity of ApoB between patients and controls. *$p < 0.01$.

arylesterase activity was also significantly lower in patients [PON in patients 118.5 ± 64.6 U/ml, in controls 188.0 ± 77.6 U/ml ($p < 0.001$); arylesterase in patients 78.9 ± 15.2 U/ml, in controls 123.7 ± 29 U/ml ($p < 0.0001$; Figure 3)].

The PON phenotype of patients with MCTD did not differ from the phenotype in healthy controls (Table 2). The AA phenotype in patients, 56.7% (controls 60%); AB in patients, 35.1% (controls 33.3%); BB in patients, 8.1% (controls 6.6%). Patients with AA phenotype had significantly less PON activity than the homozygous BB and the heterozygous AB phenotype patients (PON activity: AA, 77.3 ± 24.7 U/ml; AB, 147.6 ± 50.9 U/ml; BB, 256.8 ± 30.7 U/ml).

We analyzed the correlation between decrease of PON activity and MCTD disease status and the individual vascular alterations. The decrease of PON activity showed a negative correlation with the age of patients with MCTD ($r = –0.496$, $p < 0.05$) and the duration of MCTD ($r = –0.522$, $p < 0.001$; Table 3). We found no correlation between PON activity and corticosteroid treatment in patients. Of note, we found a significant correlation between the cardiovascular and cerebrovascular events, retinal circulatory disorder, and the decreased PON activity.

Compared to controls, patients with MCTD had elevated levels of CRP, the marker of continuous inflammation and damage of the vessels. Further, the serum levels of AECA, vWF antigen, and TM were also increased (Table 4).

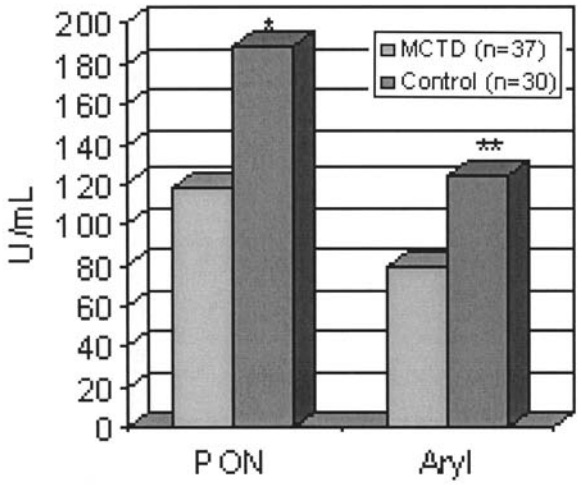

Figure 3. PON and arylesterase activity in the sera of patients with MCTD. The quantity of PON was decreased in sera of patients compared to controls, and arylesterase activity was also significantly lower in patients. *$p < 0.001$; **$p < 0.0001$.

Table 3. Correlation between paraoxonase (PON) activity and MCTD status.

Measure	Correlation with PON Activity	p
Age at survey	R = –0.496	< 0.05*
Duration of MCTD	R = 0.522	< 0.001*
Corticosteroid therapy	OD 0.2749	NS†
Cardiovascular event	OD 7.5	< 0.001†
Central nervous system abnormality	OD 18.9	< 0.001†
Circulatory disturbances of retina	OD 12.3	< 0.01†

* Correlation; † chi-square. NS: nonsignificant.

The decrease of PON activity showed a negative correlation with the high serum concentration of AECA and elevated serum levels of TM and vWF antigen (Figures 4, 5, and 6, respectively).

DISCUSSION

A significant factor in preventing lipid peroxidation is the HDL molecule and associated enzymes[23]. Produced by the liver, PON binds to the HDL with the help of ApoA1 and prevents LDL oxidation and the formation of lipoperoxides[24].

It has been reported that not only PON but also ApoA1 is necessary for the prevention and removal of lipid peroxidation. We were the first to study and verify in a large group of patients with MCTD that the decreased PON and ApoA1 activity in MCTD and the changes of serum total cholesterol may be involved in the development of atherosclerotic events in MCTD.

PON is an ester hydrolase produced by the liver and it binds to ApoA1. One natural physiological function of PON appears to be the metabolization of toxic oxidized lipids of both LDL particles and HDL particles. Paragh, *et al* observed decreased PON activity in patients with chronic renal disease following kidney transplant[25]. Tsuzura, *et al* found that serum PON activity was lower in diabetes mellitus; further, patients with diabetic vascular complications had a more significant decrease of PON activity[26]. The decreased PON level and activity in our patients with MCTD correlated closely with the age of patients and the duration of disease. Seres, *et al* were the first to report that PON activity decreased with aging[27]. Decreasing PON activity was observed in sera of patients with

Table 2. Phenotype and activity of paraoxonase (PON) in patients with MCTD.

PON Phenotype	Controls, n = 30 (%)	PON Activity in Controls, U/ml, n = 30, %	Patients, n = 37 (%)	PON Activity in Patients, U/ml
AA	18 (60)	151.3 ± 41.3	21 (56.8)	77.3 ± 24.7[a]
AB	10 (33.3)	211.8 ± 55.3	13 (35.1)	147.6 ± 50.9[b]
BB	2 (6.7)	400.0 ± 8.4	3 (8.1)	256.8 ± 30.7[c]

Statistical significance: a-b, $p < 0.001$; a–c, $p < 0.001$; b–c, $p < 0.01$.

Table 4. Levels of CRP and factors indicating endothelial cellular activation [antiendothelial cell antibody, von Willebrand factor (vWF) antigen, and thrombomodulin] in sera of patients with MCTD.

Laboratory Results	Patients, n = 37	Controls, n = 30	p
CRP, mg/l	17.3 ± 14.3	1.8 ± 0.97	< 0.01
Antiendothelial cell antibody, U/ml	36.8 ± 32.8	17.7 ± 10.2	< 0.001
Thrombomodulin, ng/ml	12.27 ± 11.7	3.2 ± 2.6	< 0.001
vWF antigen, %	172.6 ± 68	107.4 ± 31	< 0.001

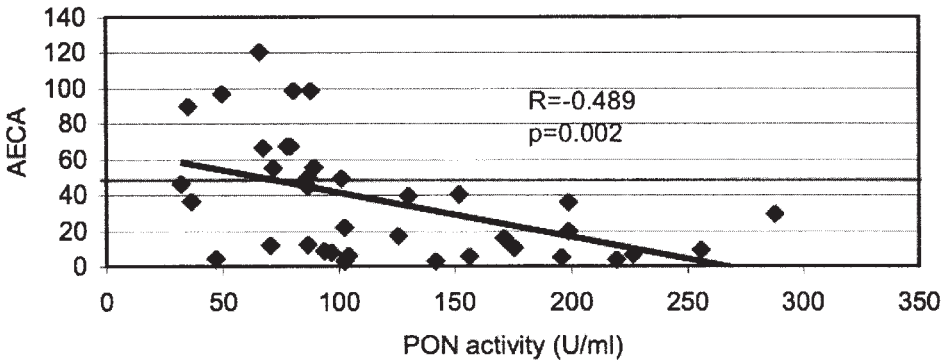

Figure 4. Correlation between PON activity and level of antiendothelial cell antibodies (AECA) in sera of patients with MCTD.

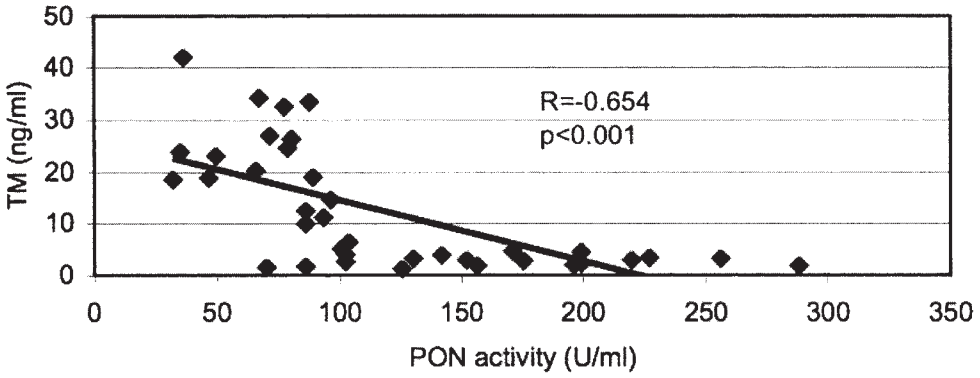

Figure 5. Correlation between PON activity and level of thrombomodulin (TM) in sera of patients with MCTD.

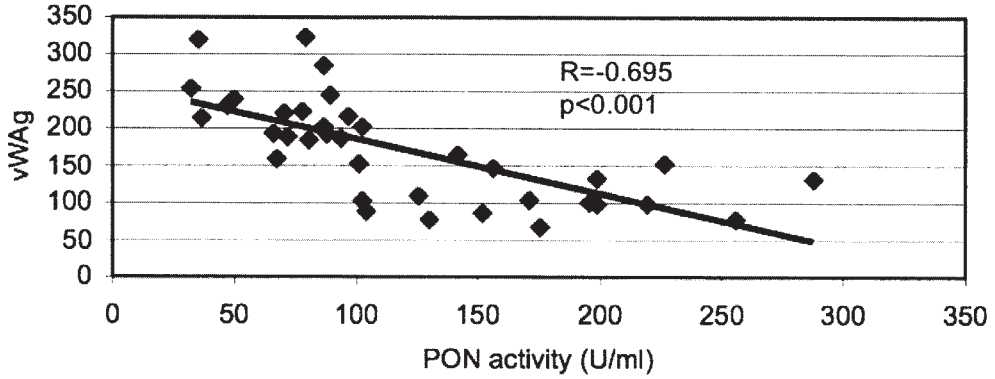

Figure 6. Correlation between PON activity and level of vWF antigen in sera of patients with MCTD.

SLE[28,29]. Tanimoto, *et al* found decreased PON activity in patients with RA; additionally, they observed low ApoA1 levels, similar to our results derived from patients with MCTD[30].

The mechanism of decrease in serum PON activity in MCTD patients remains unclear. Inflammatory mediators present in the sera of patients with MCTD might affect and modify the activity of PON. However, decreased PON production by the liver and the consequent decrease in PON activity cannot be ruled out.

The mean age of our patient group was nonsignificantly higher than that for the controls. Our data and previous findings indicated PON activity depends on age, thus we performed further statistical analyses. Both a model stratified for age and a multiple regression analysis showed that PON activity was significantly reduced, independently of age, in the MCTD group compared to controls. Moreover, the other variables, TM, vWF antigen, and AECA, showed strong correlations with PON activity, independently from age (data not shown). These analyses emphasized that the almost 5-year difference between patients and controls could not cause this reduction.

ApoA1 is essential for association of PON1 with HDL and also important for its optimal activity[31]. The low arylesterase activity of the sera from patients with MCTD implies that ApoA1 has a diminished potential to stabilize PON to the HDL. Abe, *et al* reported anti-ApoA1 antibodies in patients with SLE, and a decrease of ApoA1 concentration was also found in our patients with MCTD. Further studies are needed to determine the mechanism of the reduction of ApoA1 in MCTD.

PON is an antioxidant enzyme that is not exclusively present in the liver, but can be found in endothelial cells of the arterial walls and in smooth muscle cells as well. The presence of PON in arterial wall protects LDL cholesterol from oxidation[32]. The primary site of inflammation in patients with MCTD is in the vessel walls. The presence of AECA may activate/damage the endothelial cells; consequently, monocytes and granulocytes migrate through the activated endothelium into the tissues[33,34]. The decrease of PON activity showed strong association with the elevated serum levels of vWF antigen and TM, which are the markers of vascular damage[35,36].

Interestingly, the decreased activity of PON in patients with MCTD showed a strong association with cardiovascular events — myocardial infarction, angina, ischemic heart disease, and vascular demyelinization abnormalities verified by CT or MRI.

Genetic polymorphism also affects the concentration of PON[37-39]. Homozygous BB individuals had higher PON concentrations compared to homozygous AA individuals, while the heterozygous AB individuals had values between the two. We found 56.7% of the patients with MCTD in our study had the homozygous aa phenotype, therefore these patients had decreased PON activity *ab ovo*. However, the phenotypes of individuals in the control group were the same as those of the patients with MCTD; further, the activity and concentration of PON was significantly lower in patients with MCTD compared to controls. Accordingly, the very low PON activity observed in patients with MCTD cannot be explained solely by genetic factors.

The role of corticosteroids may not be completely straightforward; low doses may have a beneficial antiinflammatory role, and 10 mg/day corticosteroid therapy does not have a significant effect on the serum cholesterol level, while higher doses may exacerbate metabolic factors[40,41]. The effect of corticosteroids in response to PON concentration and activity also remains controversial. In our study the mean dosage of corticosteroid therapy was 8.2 mg/day, and we found no difference between the values of patients taking corticosteroids and those who did not receive corticosteroid therapy.

PON is an enzyme in conjunction with HDL and is known to have a protective role against atherosclerosis. The mechanism of how PON activity decreases in patients with MCTD is not known. PON is synthesized in the liver, but is also expressed in the arterial wall. Serum PON activity, ApoA1 reduction, and the increase in LDL oxidation leads to structural and functional changes of the blood vessel wall, which results in the TM and vWF antigen expression characterstic of patients with MCTD. Our results support the idea that in MCTD there is an increased risk for atherosclerosis. In addition to elevated levels of cholesterol and triglyceride, reduced PON levels and PON activity may play a crucial role in the development of atherosclerosis. Thus, there is diminished elimination of free radicals produced during the increased oxidative processes in patients with MCTD, which may worsen and maintain the damage to endothelial cells.

REFERENCES

1. Alves JD, Ames PRJ, Donohue S, et al. Antibodies to high-density lipoprotein and ß2-glycoprotein I are inversely correlated with paraoxonase activity in systemic lupus erythematosus and primary antiphospholipid syndrome. Arthritis Rheum 2002;46:2686-94.
2. van Leuven SI, Kastelein JJP, D'Cruz DP, Hughes GR, Stroes ES. Atherogenesis in rheumatology. Lupus 2006;15:117-21.
3. Goodson NJ, Wiles NJ, Lunt M, Barret EM, Silman AJ, Symmons DP. Mortality in early inflammatory polyarthritis: cardiovascular mortality is increased in seropositive patients. Arthritis Rheum 2002;46:2010-9.
4. Orem A, Deger O, Cimsit G, et al. Plasma lipoprotein(a) and its relationship with disease activity in patients with Bechet's disease. Eur J Clin Chem Clin Biochem 1995;33:473-8.
5. Cervera R, Khamashata MA, Font J, et al. Morbidity and mortality in systemic lupus erythematosus during a 5-year period. A multicenter prospective study of 1000 patients. European Working Party on Systemic Lupus Erythematosus. Medicine (Baltimore) 1999;78:167-75.
6. Ward MM. Premature morbidity from cardiovascular and cerebrovascular diseases in women with systemic lupus erythematosus. Arthritis Rheum 1999;42:338-46.
7. Jara LJ, Medina G, Vera-Lastra O, Amigo MC. Accelerated atherosclerosis, immune response and autoimmune rheumatic diseases. Autoimmun Rev 2006;5:195-201.

8. Matsuura E, Kobayashi K, Koike T, Shoenfeld Y. Autoantibody-mediated atherosclerosis. Autoimmun Rev 2002;1:348-53.
9. Mackness B, Durrington PN, Mackness MI. Human serum paraoxonase. Gen Pharmacol 1998;31:329-36.
10. Chait A, Han CYH, Oram JF, Heinecke JW. Lipoprotein-associated inflammatory proteins: markers or mediators of cardiovascular disease? J Lipid Res 2005;46:389-93.
11. Sharp GC, Irvin WS, Tan EM, Gould RG, Holman HR. Mixed connective tissue disease: an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). Am J Med 1972;52:148-59.
12. Hoffman RW, Greidinger EL. Mixed connective tissue disease. Curr Opin Rheumatol 2000;12:386-90.
13. Aringer M, Steiner G, Smolen JS. Does mixed connective tissue disease exist? Yes. Rheum Dis Clin N Am 2005;31:411-20.
14. Sullivan WD, Hurst DJ, Harmon CE, Esther JH. A prospective evaluation emphasizing pulmonary involvement in patients with mixed connective tissue disease. Medicine 1984;63:92-107.
15. Hassan BA, Ronnelid J, Gunnarsson I, Karlsson G, Berg L, Lundberg I. Increased serum level of immunoglobulins, C-reactive protein, type 1 and type 2 cytokines in patients with mixed connective tissue disease. J Autoimmun 1998;5:503-8.
16. Hassan BA, Gunnarsson I, Karlsson G, Klareskog L, Forslid J, Lundberg IE. Longitudinal study of interleukin-10, tumor necrosis factor-alpha, anti-U1-snRNP antibody levels and disease activity in patients with mixed connective tissue disease. Scand J Rheumatol 2001;5:282-9.
17. Bodolay E, Aleksza M, Antal-Szalmás P, et al. Serum cytokine levels, type 1 and intracellular T cell cytokine profiles in mixed connective tissue disease. J Rheumatol 2002;29:2136-42.
18. Bodolay E, Csíp I, Gál I, et al. Antiendothelial cell antibodies in mixed connective tissue disease: frequency and association with clinical symptoms. Clin Exp Rheumatol 2004;22;409-15.
19. Jang JJ, Olin JW, Fuster V. A teenager with mixed connective tissue disease presenting with an acute coronary syndrome. Vasc Med 2004;9:31-4.
20. Kanazawa M, Wada Y, Ohno T, et al. Mixed connective tissue disease associated with anti-neutrophil cytoplasmic antibodies against proteinase-3 and systemic atherosclerosis: a case report. Clin Rheumatol 2004;23:456-59.
21. Alarcon-Segovia DA, Villareal M. Classification and diagnostic criteria for mixed connective tissue disease. In: Kasukawa R, Sharp GC, editors. Mixed connective tissue disease and anti-nuclear antibodies. Amsterdam: Excerpta Medica; 1987:33-40.
22. Paragh G, Seres I, Harangi M, et al. The effect of micronised fenofibrate on paraoxonase activity in patients with coronary heart disease. Diabetes Metab 2003;29:613-8.
23. Norata GD, Pirillo A, Catapano AL. Modified HDL: biological and physiological consequences. Nutr Metab Cardiovasc Dis 2006;16:371-86.
24. Getz GS, Reardon CA. Paraoxonase, a cardioprotective enzyme: continuing issues. Curr Opin Lipidol 2004;15:261-7.
25. Paragh G, Seres I, Balogh Z, et al. The serum paraoxonase activity in patients with chronic renal failure and hyperlipidaemia. Nephron 1998;80:166-70.
26. Tsuzura S, Ikeda Y, Suehiro T, et al. Correlation of plasma oxidized low-density lipoprotein levels to vascular complications and human paraoxonase in patients with type 2 diabetes. Metabolism 2004;53:297-02.
27. Seres I, Paragh G, Deschene E, Fulop T Jr, Khalil A. Study of factors influencing the decreased HDL associated PON1 activity with aging. Exp Gerontol 2003;39:59-6.
28. Dinu AR, Merril JT, Shen C, Antonov IV, Myones BL, Lahita RG. Frequency of antibodies to the cholesterol transport protein apolipoprotein A1 in patients with SLE. Lupus 1998;7:355-60.
29. Abe H, Tsuboi N, Suzuki S, Sakuraba H, Takanashi H, Tahara K, Tonozuka N, Hayashi T, Umeda M. Anti-apolipoprotein A-I autoantibody characterisation of monoclonal autoantibodies from patients with systemic lupus erythematosus. J Rheumatol 2001;28:990-5.
30. Tanimoto N, Kumon Y, Suehiro T, et al. Serum paraoxonase activity decreases in rheumatoid arthritis. Life Sci 2003;72:2877-85.
31. Mackness MI, Durrington PN. High density lipoprotein, its enzymes and their potential to influence lipid peroxidation. Atherosclerosis 1995;115:243-53.
32. Mackness B, Hunt R, Durrington PN, Mackness MI. Increased immunolocalisation of paraoxonase, clusterin and apolipoprotein A1 in the human artery wall with progress of atherosclerosis. Arterioscler Thromb Vasc Biol 1997;17:1233-8.
33. Sasaki N, Kurose A, Inoue H, Sawai T. A possible role of anti-endothelial cell antibody in the sera of MCTD patients on pulmonary vascular damage relating to pulmonary hypertension. Ryumachi 2002;42:885-94.
34. Nishimaki T, Aotsuka S, Kondo H. Immunological analysis of pulmonary hypertension in connective tissue diseases. J Rheumatol 1999;26:2357-62.
35. Salomaa V, Matei C, Aleksic N, et al. Soluble thrombomodulin as a predictor of incident coronary heart disease and symptomless carotid artery atherosclerosis in the Atherosclerosis Risk in Communities (ARIC) Study: A case-cohort study. Lancet 1999;353:1729-34.
36. Wu KK. Soluble thrombomodulin and coronary heart disease. Curr Opin Lipidol 2003;14:373-5.
37. Pasdar A, Ross-Adams H, Cumming A, et al. Paraoxonase gene polymorphism and haplotype analysis in a stroke population. BMC Med Genet 2006;7:28.
38. Li HL, Liu DP, Liang CC. Paraoxonase gene polymorphism, oxidative stress, and diseases. J Mol Med 2003;81:766-9.
39. Association between PON1 polymorphisms, PON activity and diabetes complications. J Diabetes Complications 2006;20:322-8.
40. Doria A, Sarzi-Puttini P, Shoenfeld Y. 2nd Conference on Heart, Rheumatism and Autoimmunity, Pescara, Italy, May 19-20, 2005. Autoimmun Rev 2006;5:55-63.
41. Lahita RG, Rivkin E, Cavanagh I, Romano P. Low levels of total cholesterol, high density lipoprotein A1 in association with anticardiolipin antibodies in patients with systemic lupus erythematosus. Arthritis Rheum 1993;36:1566-74.