# Letters to the Editor

## Acupuncture enhances superoxide dismutase (SOD) activity in the serum of rheumatoid arthritis patients

Sirs,

Acupuncture has been sought by patients with rheumatoid arthritis (RA) for symptomatic treatment (1, 2). However, the underlying mechanism responsible for the therapeutic effect of acupuncture is not well known. RA is a chronic autoimmune disease, principally characterized by synovial inflammation of the joints. In pathogenesis of RA, the formation of oxygen free radicals (OFRs) is an essential process (3, 4). Antioxidants such as superoxide dismutase (SOD) reduce the cell damage caused by OFR, and it is suggested that the increased antioxidant activity including SOD relieves the symptoms of RA (5). Therefore, we hypothesized that acupuncture might modulate oxidative stress by enhancing the activity of antioxidants in RA patients, and investigated whether acupuncture could affect the activities of antioxidants such as SOD, catalase and total antioxidant status (TAS) in the serum of RA patients. This is a follow-up analysis of the previously reported clinical study (1).

Since a formal ethics committee had not been established at our institution at the time of this study, we assessed the appropriateness of the protocol by consulting the senior members of the college staff, including medical doctors, a nurse and a priest. Twenty-five patients who met the American College of Rheumatology criteria for RA attended with informed consent. Among those patients, 23 completed 14 sessions of partially individualized acupuncture treatment for 6 weeks. A total of 21 patients were included in this study because two blood samples from two patients were not separated properly. Changes in the activities of SOD, catalase and TAS were assessed at baseline and after 6 weeks of treatment. For control purposes, previously reported swollen joint counts (1) were used as covariates.

After the acupuncture treatment, the activities of SOD and catalase significantly increased ($p<0.01$ and $p<0.05$, respectively), but there was no change in TAS. We further investigated whether the changes in swollen joint counts were correlated with the changes in SOD, catalase or TAS in the RA patients using Spearman correlation coefficients. Interestingly, the results showed that the decrease in the number of swollen joints was significantly correlated with the increased SOD activity (r=0.44, $p<0.05$), but not with the activity of catalase ($p=0.196$) or TAS ($p=0.288$). To observe the relationship between the changes in symptoms and SOD activity more clearly, we divided all of the participants into two groups: responders (at least 50% reduction in swollen joint counts) or non-responders (less than 50% reduction in swollen joint counts), and compared the changes in SOD, catalase and TAS between two groups. The responders showed significantly greater changes in the activity of SOD ($p<0.01$) than the non-responders, but there were no significant differences in the changes of the catalase activity and TAS between the groups (Table I). The sensitivity analysis indicated that the change of SOD was not influenced by medication.

These findings suggested that the acupuncture treatment induced the increased activities of SOD and catalase in the serum of RA patients and that the reduction in the number of swollen joints was significantly related to the upregulation of SOD activity. Although a variety of factors such as susceptibility genes, disease-causing immune cells, cytokine and signal transduction networks are involved in the pathogenesis of RA (6), our results could participate as valuable pieces in unscrambling the puzzle to the yet indistinct mechanism of acupuncture treatment in RA. Since this study has limitations in the small sample size and no control group, more rigorous studies with a placebo control group are warranted to confirm these findings. This study was supported by the AMMR Project of KIOM and the SRC program of KOSEF (R11-2005-014).

**Table I.** Baseline characteristics and the comparisons of SOD, catalase and TAS before and after 6 week acupuncture treatment (n=21).

| Baseline | Week 6 | *p*-value | | Subgroup analysis | | |
|---|---|---|---|---|---|---|
| | | | | Subgroup* | Changes | *p*-value |
| Age-yrs | 52.4 (7.6) | | | | | |
| BMI | 22.0 (3.5) | | | | | |
| Female (%) | 85.7 % | | | | | |
| Acupuncture naive (%) | 28.6 % | | | | | |
| Medication (%) | Yes: 57.1 %<br>No: 42.9 % | | | | | |
| Duration of disease-yrs | 8.7 (7.9) | | | | | |
| Swollen joint count | 4.5 (3.0) | 1.0 (1.2) | <0.01 | A | - 4.3 (2.6) | <0.01 |
| | | | | B | - 0.2 (0.4) | |
| SOD (U/ml) | 1.8 (2.3) | 4.1 (1.9) | <0.01 | A | 2.8 (2.4) | <0.01 |
| | | | | B | 0.3 (0.6) | |
| Catalase (U/ml) | 60.2 (9.0) | 64.3 (11.7) | <0.05 | A | 5.4 (8.6) | NS |
| | | | | B | -0.2 (4.8) | |
| TAS (mmol/ml) | 1.0 (0.2) | 1.1 (0.3) | NS | A | 0.1 (0.3) | NS |
| | | | | B | - 0.0 (0.1) | |

Data were represented as mean (SD). *A: Responder group (n=16); B: Non-responder group (n=5). Responders were defined as patients in whom the change in the swollen joint counts from baseline was higher than 50%. Wilcoxon's signed-ranks test was used for before-and-after comparisons, and comparisons between two groups were made using the Mann-Whitney U-test. BMI: body mass index; NS: not significant.

S.T. KIM[1,23], *KMD, PhD*
Y.J. KIM[2], *MSc*
J.Y. LEE[1], *KMD, MSc*;
H. LEE[1,4], *KMD, PhD*
C.S. YIN[1], *KMD, PhD*
S.M. CHOI[5], *KMD, PhD*
Y. CHAE[1,2], *KMD, PhD*
H. LEE[1,2], *KMD, PhD*
H.J. PARK[1,2], *KMD, PhD*

*[1]Acupuncture & Meridian Science Research Center; [2]Department of Meridian & Acupoint, College of Korean Medicine, Kyung Hee University, Seoul, Republic of Korea; [3]School of Oriental Medicine, Pusan National University, Busan, Republic of Korea; [4]College of Korean Medicine, Sangji University, Wonju, Republic of Korea; [5]Department of Medical Research, Korea Institute of Oriental Medicine, Daejeon, Republic of Korea.*

*Address correspondence to: Dr. Hi-Joon Park, Department of Meridian and Acupoint, College of Korean Medicine, Kyung Hee University, 1 Hoegi-dong, Dongdaemoon-gu, Seoul 130-701, Republic of Korea. E-mail : acufind@khu.ac.kr*

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### References

1. LEE H, LEE JY, KIM YJ *et al.*: Acupuncture for symptom management of rheumatoid arthritis: a pilot study. *Clin Rheumatol* 2008; 27: 641-5.
2. WANG R, JIANG C, LEI Z, YIN K: The role of different therapeutic courses in treating 47 cases of rheumatoid arthritis with acupuncture. *J Tradit Chin Med* 2007; 27: 103-5.
3. HITCHON CA, EL-GABALAWY HS: Oxidation in rheumatoid arthritis. *Arthritis Res Ther* 2004; 6: 265-78.
4. KAGEYAMA Y, TAKAHASHI M, ICHIKAWA T, TORIKAI E, NAGANO A: Reduction of oxidative stress marker levels by anti-TNF-alpha antibody, infliximab, in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2008; 26: 73-80.
5. AFONSO V, CHAMPY R, MITROVIC D, COLLIN P, LOMRI A: Reactive oxygen species and superoxide dismutases: role in joint diseases. *Joint Bone Spine* 2007; 74: 324-9.
6. WONG SH, LORD JM: Factors underlying chronic inflammation in rheumatoid arthritis. *Arch Immunol Ther Exp (Warsz)* 2004; 52: 379-88.

## Crescentic glomerulonephritis and circulating antineutrophil cytoplasmic antibodies in scleroderma: a case report and review of the literature

Sirs,

Scleroderma renal crisis (SRC) is the classic clinical feature of kidney involvement in systemic sclerosis (SSc) occurring in about 20% of patients with diffuse cutaneous involvement and characterized by a typical clinical picture and a specific histological pattern (1). Kidney involvement different from SRC has been described in 26% of a large series of SSc patients (2). SRC-unrelated hypertension and proteinuria due to D-penicillamine toxicity were two of the main causes of renal complications, but pathogenic mechanisms leading to kidney disease other than SRC remains largely unclear.

We report a case of crescentic glomero nephritis (GN) with progressive renal failure in a 56-year-old Caucasian woman, with a diagnosis of diffuse SSc Scl-70+ since 1999, with evidence of circulating perinuclear-antineutrophil cytoplasmic antibodies (p-ANCA). She was referred at the end of 2006 for recent onset of ingravescent mild dyspnea at exercise. Few months earlier, monthly iloprost infusions were started because of distal cutaneous ulcers appearance. Laboratory data showed normochromic normocitic anemia without signs of microangiopathic hemolytic anemia. Blood urea nitrogen (BUN) was 73 mg/dl and serum creatinine 1.8 mg/dl. A 24-h urine collection contained 0.8 g of protein. Blood pressure was 160/90 mm Hg. Pulmonary function tests revealed a mild reduction in forced vital capacity with a severe reduction in diffusing lung capacity of carbon monoxide. A chest high resolution computed tomography showed bilateral medium-basal honeycombing. There was no evidence of pulmonary hypertension at transthoracic echocardiography.

Treatment with angiotensin-converting enzyme inhibitol (ACE-I) was started. However, BUN and creatinine level increased up to 139 mg/dl and 2.7 mg/dl, respectively. The 24-h urinary protein excretion was 2.6g. Urinalysis showed many erythrocytes and leucocytes per hpf and some granular casts. A high titer of p-ANCA was detected by indirect immunofluorescence (IIF), confirmed at a second determination after four months. Anti-myeloperoxidase (MPO) and anti-proteinase 3 (PR3), however, resulted persistently negative by enzyme-linked immunosorbent assay (ELISA). Anti-glomerular basement membrane (GBM) antibodies were not detected. Renal ultrasound examination showed a parenchimal nephropathy with increased cortical echogenicity. Normal renal arteries with normal intraparenchimal resistances were demonstrated at echo-doppler. A kidney biopsy showed a crescentic proliferative GN with fibrosis without evidence of thrombotic microangiopathy. Arteries and arterioles showed mild intima-media fibrous thickening. Tubules were atrophic without evidence of inflammatory infiltrate in the interstitium. On IIF staining no significant immune complex deposition was revealed. Despite treatment with intravenous cyclophosphamide, her renal failure did not recovery and hemodialysis was started.

Perinuclear or atypical ANCA pattern, usually not associated to anti-PR3 or MPO antibodies, has been described in a minority of patients with systemic lupus erythematosus, rheumatoid arthritis, polymyositis and antiphospholipid syndrome (3). Interestingly, no evidence of vasculitis or renal disease was found in these patients. Perinuclear or atypical ANCA have been also reported in SSc, with a prevalence of up to 15% (4). A crescentic GN was present in some of these subjects.

Crescentic rapidly progressive GN, clinically presenting as RPGN, is classified in three major types: anti-GBM GN (I), immune-complex GN (II) and pauci-immune GN (III) (5). Type III crescentic GN is associated with circulating ANCA in approximately 80% of patients, but a possible direct pathogenetic role of ANCA in renal damage is controversial (3).

Recently, pauci-immune crescentic GN in SSc has been described and it is intriguing the fact that all cases were associated with ANCA positivity (Table I). ANCA-associated renal involvement in SSc is characterized by normal or mild elevated blood pressure values, ingravescent renal failure, active urinary sediment with casts and proteinuria (sometimes in nephrotic range). Disease duration before the onset of renal failure is usually much longer in ANCA-related GN than in typical SRC. Moreover, histopathologic findings, all characterized by crescentic GN, are very different from those found in SRC. ANCA-associated kidney involvement in scleroderma mainly occurs in Scl-70+ diffuse skin disease, but few cases in anti-centromere positive patients have been also reported. Occasionally, ANCA+ subjects with clinical features of SSc and circulating anti-DNA, anti-Sm/RNP and anti-Ro/La antibodies have been described (6, 8, 12). Of interest, none of these patients had clinical evidence of overt connective tissue disease.

Unlike other described cases, ANCA reactivity in our patient was not associated with anti-MPO. Kidney biopsy showed an advanced sclerotic stage, so that an earlier MPO detection during more active renal damage could not be ruled out. However, our findings may suggest that crescentic GN in SSc is also associated with ANCA reactive against autoantigens different from MPO.

In conclusions, the present report supports the existence of different forms of renal involvement other than SRC in scleroderma with possible different pathogenic mechanisms. Because of different therapeutic approaches, these findings may suggest the usefulness to perform renal biopsy in cases of renal impairment in SSc patients, particularly in subjects with circulating ANCA.

**Table I.** Clinical features of SSc patients with pauci-immune crescentic GN and circulating ANCA.

| Author (Ref) | Pts n. (Sex) | Age (yrs) | Skin 3 D | Disease duration | BP (mmHg) | Creat. (mg/dl) | 24h-UP (g) | AutoAb specificity |
|---|---|---|---|---|---|---|---|---|
| Endo (6) | 6 (F) | 50 mean | 3 L | 7.8 y mean | 158/86 mean | 4.8 mean | ? | Sm/RNP anti-DNA |
| Huong (7) | 1 (F) | 59 | D | 10 y | 140/80 | 7.5 | 1.0 | ACA |
| Hillis (8) | 1 (F) | 62 | L | 18 y | normal | 4.5 | ? | Ro/Scl-70 |
| Carvajal (9) | 1 (F) | 54 | D | 8 y | 150/90 | 1.7 | 1.5 | Scl-70 |
| Katrib (10) | 3 (1F,2M) | 66 mean | L | 4.5 y mean | 123/80 mean | 2.4 | ? | all ACA |
| Villaverde (11) | 1 (F) | 60 | D | 1 y | 140/80 | 2.2 | 4.3 | Scl-70 |
| Wutzl (12) | 1 (M) | 43 | D | 18 y | 135/70 | 1.8 | 3.1 | Ro/La |
| Tomioka (13) | 1 (F) | 64 | no | 30 y | 164/90 | 2.3 | 0.72 | Scl-70 |
| Herrera (14) | 1 (F) | 52 | D | 1 y | ? | 4.5 | > 3.5 | ACA |
| Kamen (15) | 1 (M) | 45 | D | 5 months | 157/98 | 5.1 | 3.8 | Scl-70 |
| Kamen (15) | 1 (M) | 19 | D | 5 y | 130/85 | 1.6 | 0.9 | Scl-70 |
| Kamen (15) | 1 (F) | 60 | D | 8 y | 118/70 | 2.2 | ? | Scl-70 |
| Cheung (16) | 1 (F) | 54 | D | 3 y | 110/60 | ↑ | ? | Scl-70 |
| Arnaud (17) | 1 (F) | 46 | D | 10 y | 104/65 | 1.33 | 0.45 | Scl-70 |

D: diffuse; L: limitate; UP: urinary proteins; BP: blood pressure.

E. Bartoloni Bocci
A. Giordano
F. Luccioli
A. Alunno
R. Gerli

*Rheumatology Unit, University of Perugia, Perugia, Italy.*

*Address correspondence to: Professor Roberto Gerli, Rheumatology Unit, Policlinico Monteluce, Università di Perugia, 06122 Perugia, Italy. E-mail: gerlir@unipg.it*

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## References


1. PENN H, HOWIE A, KINGDON E *et al*.: Scleroderma renal crisis: patient characteristics and long-term outcomes. *Q J Med* 2007; 100: 485-94.
2. STEEN V, SYZD A, JOHNSON J, GREENBERG A, MEDSGER T JR: Kidney disease other than renal crisis in patients with diffuse scleroderma. *J Rheumatol* 2005; 32: 649-55.
3. BOSCH X, GUILABERT A, FONT J: Antineutrophil cytoplasmic antibodies. *Lancet* 2006; 368: 404-18.
4. MANOLOVA I, DANCHEVA M: Antineutrophil cytoplasmic antibodies in patients with systemic sclerosis. *J Rheumatol* 2003; 30: 2078-9.
5. IZZEDINE H, CAMOUS L, DERAY G: New insight on crescentic glomerulonephritis. *Nephrol Dial Transplant* 2007; 22: 1480-1.
6. ENDO H, HOSONO T, KONDO H: Antineutrophil cytoplasmic antibodies in 6 patients with renal failure and systemic sclerosis. *J Rheumatol* 1994; 21: 864-70.
7. HUONG DL, PAPO T, GATFOSSE M, FRANCES C, GODEAU P, BEAUFILS H: Antineutrophil cytoplasmic antibodies in systemic sclerosis with renal failure. *J Rheumatol* 1995; 22: 791-2.
8. HILLIS G, KHAN I, SIMPSON J, REES A: Scleroderma, D-penicillamine treatment, and progressive renal failure associated with positive antimyeloperoxidase antineutrophil cytoplasmic antibodies. *Am J Kidney Dis* 1997; 30: 279-81.
9. CARVAJAL I, BERNIS C, SANZ P, GARCIA A, GARCIA-VADILLO A, TRAVER J: Antineutrophil cytoplasmic antibodies (ANCA) and systemic sclerosis. *Nephrol Dial Transplant* 1997; 12: 576-7.
10. KATRIB A, STURGESS A, BERTOUCH J: Systemic sclerosis and antineutrophil cytoplasmic autoantibody-associated renal failure. *Rheumatol Int* 1999; 19: 61-3.
11. VILLAVERDE V, BALSA A, CABEZAS J, FERNÀNDEZ-PRADA M, TORRE A, MOLA E: Normotensive renal failure in a patient with systemic sclerosis and p-neutrophil cytoplasmic autoantibodies which developed into Paget's disease of bone after immunosuppressive therapy. *Rheumatology* 1999; 38: 190-1.
12. WUTZL A, FOLEY R, O'DRISCOLL B, REEVE R, CHISHOLM R, HERRICK A: Microscopic polyangiitis presenting as pulmonary-renal syndrome in a patient with long-standing diffuse cutaneous systemic sclerosis and antibodies to myeloperoxidase. *Arthritis Care Res* 2001; 45: 533-6.
13. TOMIOKA M, HINOSHITA F, MIYAUCHI N, AKIYAMA Y, SAIMA S, HIROE M: ANCA-related crescentic glomerulonephritis in a patient with scleroderma without marked dermatological change and malignant hypertension. *Int Med* 2004; 43: 496-502.
14. HERRERA-ESPARZA R, AGUILAR J, SAUCEDO A, GONZÀLEZ I, LÒPEZ-ROBIES E, AVALOS-DÌAZ E: Sclerodermia with type III glomerulonephritis and MPO-ANCA antibodies in the serum. *JEADV* 2005; 19: 617-20.
15. KAMEN D, WIGLEY F, BROWN A: Antineutrophil cytoplasmic antibody-positive crescentic glomerulonephritis in scleroderma. A different kind of renal crisis. *J Rheumatol* 2006; 33: 1886-8.
16. CHEUNG G, CHEW G, WYNDHAM R, PETERS M, RIMINTON S: Myeloperoxidase-antineutrophil cytoplasmic antibody seroconversion and fulminant vasculitis in Scl-70-positive scleroderma. *Inter Med J* 2007; 37: 205-7.
17. ARNAUD L, HUART A, PLAISIER E *et al*.: ANCA-related crescentic glomerulonephritis in systemic sclerosis: revisiting the "normotensive scleroderma renal crisis". *Clin Nephrol* 2007; 68: 165-70.