Concise Report

Serum levels of 8-isoprostane, a marker of oxidative stress, are elevated in patients with systemic sclerosis

F. Ogawa, K. Shimizu, E. Muroi, T. Hara, M. Hasegawa1, K. Takehara1 and S. Sato

Objective. To determine serum levels and clinical correlation of 8-isoprostane, which is produced in vivo through free radical-catalysed peroxidation of arachidonic acid and reflects oxidative stress, in patients with systemic sclerosis (SSc).

Methods. Serum 8-isoprostane levels from 32 patients with diffuse cutaneous SSc (dSSc) and 25 patients with limited cutaneous SSc (lSSc) were examined by enzyme-linked immunosorbent assay.

Results. Serum 8-isoprostane levels were elevated in dSSc and lSSc patients by 75-fold compared with normal controls (n = 32). Serum 8-isoprostane levels correlated negatively with pulmonary function, such as percentage vital capacity and diffusion capacity for carbon monoxide, and correlated positively with renal vascular damage determined by colour flow Doppler ultrasonography. Serum 8-isoprostane levels also correlated positively with serum levels of IgG and anti-agalactosyl IgG autoantibody.

Conclusion. Increased 8-isoprostane levels correlated with the severity of pulmonary fibrosis, the extent of renal vascular damage and immunological abnormalities in SSc, suggesting that enhanced oxidative stress is related to the development of SSc.

KEY WORDS: Systemic sclerosis, Oxidative stress, 8-isoprostane, Pulmonary fibrosis, Renal vascular damage, Immunological abnormalities.

Although the pathogenesis of systemic sclerosis (SSc) remains unknown, oxidative stress has been suggested to contribute to clinical manifestations associated with SSc, such as vascular damage, fibrosis and production of autoantibodies [1–4]. To clarify the role of oxidative stress in the development of SSc, many investigators have focused on abnormalities of nitric oxide (NO) and NO synthase in SSc [5]. However, the exact status of NO generation in SSc is confusing [5], since both elevated and reduced circulating NO levels and production have been reported [6–10]. Furthermore, the physiological and pathological effects of NO are diverse and paradoxical [5]. Specifically, NO generated constitutively by endothelial cells functions as a critical vasodilator that may improve peripheral ischaemia in SSc [11]. By contrast, in certain pathological states, such as reperfusion injury secondary to Raynaud’s phenomenon, inducible NO synthase generates excessive levels of NO, which reacts with superoxides to form highly reactive hydroxyl radicals, which in turn can result in cell injury and death [12, 13].

To assess the role of free radicals in the development of SSc, more reliable markers that directly reflect free radical formation in vivo would be needed. One of these markers is 8-isoprostan e, an isoprostane, which is one of the family of eicosanoids of non-enzymatic origin produced by the random oxidation of tissue phospholipids by oxygen radicals [14]. Thus, 8-isoprostane has been proposed as a reliable biomarker of oxidative stress and antioxidant deficiency because of its biochemical stability. Indeed, increased levels of 8-isoprostane have been detected in heavy smokers [15] and could be formed following ischaemia–reperfusion sequences in angioplasty [16]. In this study, to assess the role of oxidative stress in SSc, serum 8-isoprostane levels and their clinical correlation were examined.

Patients and methods

Serum samples

Serum samples were obtained from all SSc patients who visited our scleroderma clinic over the last 7 yr. They were 57 Japanese patients with SSc (48 females, 9 males; age 49.5±17.3 yr) who fulfilled the criteria proposed by the American College of Rheumatology [17]. They were grouped according to the classification system [18]: 25 patients (23 females, 2 males; age 52.4±14.2 yr) had limited cutaneous SSc (lSSc) and 32 patients (25 females, 7 males; age 47.3±19.0 yr) diffuse cutaneous SSc (dSSc). The disease duration of lSSc and dSSc patients was 9.1±9.9 and 3.1±3.0 yr, respectively. None of SSc patients was treated with oral steroid, D-penicillamine or other immunosuppressive therapy at the evaluation. Antinuclear antibody (Ab) was determined by indirect immunofluorescence and autoantibody specificities were further assessed by enzyme-linked immunosorbent assay (ELISA) and immunoprecipitation. Anticentromere Ab was positive for 19 patients, antitopoisomerase I Ab for 25, anti-U1RNP Ab for two, anti-U3RNP Ab for one, anti-RNA...
polymerases I and III Ab for six and Th/To Ab for one. The remaining three patients were negative for autoantibody. Thirty-two healthy Japanese people of similar age and gender to patients (4 males, 28 females; age 44.2 ± 10.2 yr) were used as normal controls. Smokers were excluded in this study. Blood samples were centrifuged shortly after clot formation. All samples were stored at –80°C prior to use.

Clinical assessment
Complete medical histories, physical examinations and laboratory tests, including vital capacity (VC) and diffusion capacity for carbon monoxide (DLco), were conducted for all patients within 3 to 5 weeks after serum collection. Isolated pulmonary hypertension was defined as clinical evidence of pulmonary hypertension and increased systolic pulmonary arterial pressure (>35 mmHg) by Doppler echocardiography, in the absence of severe pulmonary interstitial fibrosis; however, there were no patients with isolated pulmonary hypertension in this study. Renal vascular damage was determined as a pulsatility index (PI) by colour flow Doppler ultrasonography of both kidneys [19]. Peripheral circulatory insufficiency was evaluated by the presence of digital pitting scars or ulcers at the first physical examination. SSc exhibits immunological abnormalities, including hyper-γ-globulinaemia and production of various autoantibodies, and anti-agalactosyl IgG Ab is one of the major autoantibodies in SSc since it is detected in more than 70% of SSc patients [20]. Serum IgG, IgM and IgA levels were measured by nephelometry (Dade Behring, Tokyo, Japan). Antigalactosyl IgG Ab was measured with a Lectin Enzyme Immunoassay kit (Eizai Co., Ltd, Tokyo, Japan) using human agalactosyl IgG as the antigen. Anticentromere and antitopoisomerase I Ab levels were measured with ELISA using human recombinant centromere and topoisomerase I protein, respectively (Medical & Biological Laboratories, Nagoya, Japan).

The results of clinical assessments were as follows: % predicted VC (mean ± S.D.), 93.8 ± 24.7% (normal value >80%); % predicted DLco, 59.3 ± 17.3% (>75%); PI, 1.26 ± 0.18 (<1.38); IgG, 321.6 ± 143.9 mg/dl (110–410); IgM, 202.4 ± 127.3 mg/dl (male 33–190, female 46–260); anti-agalactosyl IgG Ab levels, 20.5 ± 21.2 AU/ml (<6.0); anticentromere Ab levels, 164.8 ± 31.5 U/ml (<10.0); antitopoisomerase I Ab levels, 195.0 ± 81.4 U/ml (<16.0); erythrocyte sedimentation rate, 16.6 ± 12.6 mm/1 h (male 2–10, female 3–15); C-reactive protein, 0.33 ± 0.65 mg/dl (<0.17). Mean autoantibody levels were calculated only from patients positive for each autoantibody. The protocol was approved by local ethical committee of Kanazawa University School of Medicine and Kanazawa University Hospital, and informed consent was obtained from all patients according to the declaration of Helsinki.

ELISA
Specific ELISA kits were used for measuring serum levels of 8-isoprostane (Cayman, MI, USA), according to the manufacturer’s protocol. Each sample was tested in duplicate. Regarding the reproducibility, we measured 8-isoprostane levels more than twice in seven serum samples; as a result, the mean percentage coefficient of variation was 3.6%. Furthermore, to assess diurnal variation, we examined 8-isoprostane levels at 9 a.m., 0 p.m. and 5 p.m. in eight healthy persons; however, there was no significant difference.

Statistical analysis
Statistical analysis was performed using the Mann-Whitney U-test, Bonferroni’s test and Spearman’s rank correlation.

Results
Serum 8-isoprostane levels in SSc
Serum 8-isoprostane levels in SSc patients (median 441 pg/ml; range 13–154 879 pg/ml) were significantly elevated by 75-fold compared with those in normal controls (6; 2–34; P < 0.001; Fig. 1). Patients with dSSc (452; 13–154 879) had significantly higher 8-isoprostane levels than normal controls (P < 0.001). Similarly, ISSc patients (441; 41–34 058) exhibited elevated 8-isoprostane levels relative to normal controls (P < 0.001). Serum 8-isoprostane levels were similar between dSSc and ISSc patients. Values higher than the mean ± 3 S.D. of the control serum samples were considered to be elevated in this study. Remarkably, 99% (56/57) of SSc patients exhibited elevated 8-isoprostane levels, while none of the healthy individuals showed increased levels. Thus, almost all SSc patients exhibited elevated 8-isoprostane levels that could discriminate SSc patients from normal controls.

Clinical correlation of serum 8-isoprostane levels
We next evaluated clinical the association of 8-isoprostane levels in SSc. Serum 8-isoprostane levels correlated inversely with %VC (r = –0.42, P < 0.01) and %DLco (r = –0.449, P < 0.01; Fig. 2). Furthermore, there was a positive association between 8-isoprostane levels and renal vascular resistance (r = 0.53, P < 0.01), which was determined as the PI value in the renal interlobar arteries by colour-flow Doppler scans. However, 8-isoprostane levels were similar in 24 SSc patients with digital pitting scars/ulcers and 33 patients without that complication (367 pg/ml; 41–154 879 pg/ml vs 497; 13–15 390, P = 0.63). Serum 8-isoprostane levels correlated positively with serum levels of IgG (r = 0.43, P < 0.001), IgA (r = 0.44, P < 0.001) levels and anti-agalactosyl IgG Ab (r = 0.60, P < 0.001), while they did not correlate with serum levels of IgM (r = 0.09, P = 0.48). Anticentromere and antitopoisomerase I Ab levels were not correlated with 8-isoprostane levels (r = –0.50, P = 0.47 and
Discussion

The present study is the first to reveal that serum 8-isoprostane levels were elevated by 75-fold in SSc patients, suggesting that oxidative stress levels are enhanced in SSc. Previous studies showed that 8-isoprostane levels were increased in urine samples [21] and bronchoalveolar lavage (BAL) samples from SSc patients [22], although the increase was only two- to fivefold. More importantly, 99% of SSc patients exhibited increased serum 8-isoprostane levels, while none of the healthy persons had elevated levels. In this study, the prevalence of dSSc was higher than that of ISSc, which was not consistent with actual subset prevalence. The reasons for the difference are not clear; however, it may be due to racial and regional differences, since the prevalence of some clinical manifestations and disease severity has been shown to have a racial difference [23]. Nonetheless, serum 8-isoprostane levels were elevated in dSSc and ISSc to a similar extent. These results indicate that an elevated 8-isoprostane level is a common feature in SSc and can discriminate SSc patients from normal persons. Furthermore, increased serum 8-isoprostane levels were associated with the severity of lung fibrosis and the extent of vascular damage. This suggests that 8-isoprostane is a useful serological marker for evaluating oxidative injury and disease severity in SSc.

In this study, serum 8-isoprostane levels correlated with renal vascular damage in SSc. Vascular endothelial dysfunction is one of the central events in SSc, and cold- and stress-induced vasospasm (Raynaud’s phenomenon) is the most characteristic sign that reflects this dysfunction. Raynaud’s phenomenon occurs not only in the fingers and toes but also in the internal organs, such as the kidney [24]. Ischaemia and reperfusion injury following Raynaud’s phenomenon can generate reactive oxygen species that may result in vascular endothelial damage [12, 13]. In addition, 8-isoprostane itself is a potent vasoconstrictor, has platelet pro-aggregant functions and stimulates endothelial cells to bind monocytes, which may promote vascular obliteration, inflammation and spasm. Therefore, the finding that serum 8-isoprostane levels correlated with renal vascular resistance in SSc suggests that excessive oxidative stress is related to vascular damage in SSc.

A previous study showed that 8-isoprostane levels in BAL from SSc patients with lung fibrosis did not correlate with lung function [22]. The authors concluded that impairment in lung function tests is the result of previous lung damage, while the BAL 8-isoprostane levels are likely to reflect the current pathological situation [22]. By contrast, in this study serum 8-isoprostane levels correlated inversely with pulmonary function. Since the increase in serum 8-isoprostane levels (75-fold) was much greater than that in BAL 8-isoprostane levels (4.5-fold) in SSc, serum 8-isoprostane levels may be result of the accumulation of 8-isoprostane generated in internal organs, such as lung, and could reflect previous lung damage in SSc. These results suggest that serum 8-isoprostane level is a useful serological marker for the severity of lung fibrosis in SSc.

It has been hypothesized that in SSc, ischaemia–reperfusion injury due to Raynaud’s phenomenon can generate reactive oxygen species that induce autoantigen fragmentation and cryptic epitope expression, leading to autoantibody production [25]. However, in this study, serum 8-isoprostane levels did not correlate with levels of anticentromere Ab or antitopoisomerase I Ab. Nonetheless, serum 8-isoprostane levels correlated with anti-α-galactosyl IgG Ab levels, suggesting that oxidative stress may contribute to autoantibody production in SSc. Furthermore, the finding that there was a significant correlation between serum Ig levels and 8-isoprostane levels suggests that oxidative stress is related to the abnormalities of humoral immunity in SSc.

The authors have expressed no conflicts of interest.

Reference


