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Role of the free radical release process in the pathogenesis of morphea in contrast to systemic sclerosis

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Abstract The objective of this study was to assess the importance of the free radical release process in the pathogenesis of localized scleroderma and compare it with that in systemic sclerosis. The study was conducted on 20 randomly collected cases of morphea (4 single plaque, 7 linear, and 9 disseminated), 16 cases of systemic sclerosis, and 10 age- and sex-matched healthy volunteers. Blood samples and homogenized skin biopsies from lesional and nonlesional skin of patients and controls were examined for superoxide dismutase (SOD) activity using spectrophotometric assay, and for lipid peroxide level using the thiobarbituric acid assay. Morphea and systemic sclerosis cases showed significant elevation of blood, lesional, and nonlesional skin lipid peroxide levels and SOD activity compared with normal controls. When each of the subtypes of morphea were compared with the controls, a significant elevation of SOD was found in lesional skin in all groups, in plasma of linear and disseminated morphea, and in nonlesional skin of cases of disseminated morphea. A comparison of systemic sclerosis and morphea cases revealed no significant differences in blood or tissue SOD activity or lipid peroxide level. In both groups, the degree of skin induration could be correlated with changes in lesional SOD activity and lipid peroxide levels, respectively, but no correlation could be found between SOD or lipid peroxide and antinuclear antibody titer. The free radical release process is as important in the pathogenesis of morphea as it is in systemic sclerosis, where it appears to be involved in the development of skin induration.

Key words Free radicals · Lipid peroxidation · Morphea · Superoxide dismutase (SOD) · Systemic sclerosis

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Introduction

The etiology and pathogenesis of scleroderma are essentially unknown, but vascular damage, disturbed collagen formation, and abnormalities in the immune system have all been strongly incriminated.[1] The involvement of mast cells and cytotoxic agents affecting the microvasculature has also been suggested.[2] Little is known about the factor or agent that initiates the series of pathologic processes that lead to the sclerotic process.[1] Murrel[3] hypothesized that the generation of free radicals could be a common mechanism through which different etiological agents can provoke scleroderma in genetically predisposed individuals.

Oxygen free radicals in high concentrations inhibit collagen formation and even cause its degradation.[4] Conversely, the repeated release of free radicals in low concentrations stimulates fibroblast proliferation with narrowing of the vessel wall, ischemia, and the release of more free radicals.[5] It was therefore suggested that free radicals can cause sclerosis either through direct tissue damage leading to vascular endothelial necrosis,[3] or through the chronic stimulation of collagen formation.[5]

Patients with systemic sclerosis were found to have a greater oxidative metabolic activity in whole blood and polymorphonuclear leukocytes (PNL),[6] as well as increased susceptibility to oxidative DNA damage,[7] and oxidation of low-density lipoproteins (LDL).[8] This may be due to a deficiency of micronutrient antioxidants such as ascorbic acid and selenium,[9] or increased superoxide production as detected by elevated plasma superoxide dismutase (SOD) (an

important antioxidant enzyme) activity in patients with systemic sclerosis.[10] It has been suggested that metal-catalyzed oxidation reactions play a role in the early pathogenesis of the disease.[11] The role of the free radical release process has also been implicated in cases of scleroderma-like syndromes caused by environmental agents such as silica, bleomycin, alcohol, and toxic oil.[12] Some authors suggest that the release of oxygen free radicals and the associated damage to the vascular endothelium could be the primary event, which is followed by stimulation of the immune system.[3] Others suggest that in those cases their release is induced by ischemia caused by vascular damage, or due to an altered immune function.[5] An increased concentration of soluble immune activation markers, such as soluble interleukin-2 (sIL-2) receptor and gamma interferon in systemic sclerosis patients,[13] stimulates endothelial cell production of nitric acid and neopterin, the cytotoxic effect of which enhances the free radical-mediated effects.[14]

Skin changes in localized scleroderma or morphea show a marked histological and electron microscopic resemblance to systemic sclerosis apart from a lack of involvement of the muscle capillaries in morphea. The two disorders rarely occur together. Morphea cases may occasionally reveal Raynaud's phenomenon, gastrointestinal manifestations, and nail-fold capillaroscopy changes similar to those of systemic sclerosis.[15] Autoimmunity, disturbed fibroblast functions, and changes in small dermal blood vessels are common factors in the pathogenesis of the two conditions.[1] Since the role of free radicals is strongly suggested in the pathogenesis of systemic sclerosis, we would also expect them to be involved in the pathogenesis of morphea.

In this work, the role of the free radical release process in morphea was studied and compared with that in systemic sclerosis. The blood, lesional, and nonlesional skin SOD activity and lipid peroxide levels were measured to determine the degree of free radical production and to asses the degree of free radical-mediated tissue damage in both groups. Antinuclear antibody (ANA) positivity and its titer were measured to detect the relation between the free radical release and the process of autoantibody production in both diseases.

Patients and methods

Two groups of patients were enrolled in the study. The first group included 16 cases of systemic sclerosis (14 female, 87.5%) diagnosed according to the criteria of the American Rheumatism Association.[16] This group was subdivided according to clinical type into acrosclerosis (14 cases) and progressive systemic sclerosis (2 cases). Twelve cases had systemic sclerosis only, and four had other collagen diseases in addition to systemic sclerosis: two had polymyositis and two had rheumatoid arthritis. All patients had Raynaud's phenomenon, 11 (68.8%) had esophageal dysmotility, 4 (25%) had telangiectasia, 10 (62.5%) had dyspnea on exertion, and 3 (18.8%) had pulmonary hypertension. Their drug treatments included nifedipine (3 patients), diltiazem (2 patients), and methotrexate (3 patients).

The second group included 20 cases of morphea (16 female, 80%). This group was further subdivided according to clinical type into single-plaque morphea (4 cases), linear morphea (7 cases), and disseminated morphea (9 cases).

Each case had a clinical examination and a complete medical history was taken. The degree of skin induration was assessed by rating the thickness of the skin on a scale of 0–3[17] in all the lesions of morphea cases, and in at least four sites in cases of systemic sclerosis. A rating of 0 meant normal, while 1 meant minimal involvement, 3 meant severe involvement, and 2 was intermediate between 1 and 3.

Ten healthy volunteers served as a control group. A serum sample was collected from each patient and control and examined for ANA and its titer by immunofluorescence,[18] as well as for lipid peroxide level. In addition, plasma samples were examined for SOD activity. Two skin biopsies were taken from each patient (one from lesional and one from nonlesional skin), and one biopsy was taken from the covered skin of normal controls. All the skin biopsies were examined for SOD activity and lipid peroxide levels. Written informed consent was obtained from all patients and controls.

Preparation of samples

Skin biopsy

Skin biopsies were taken with a 4-mm punch from the affected and normal covered skin of patients and the normal covered skin of controls.[19] The biopsy was washed in ice-cold 0.25 M sucrose and weighed. It was then homogenized in 0.25 M sucrose in a homogenizer. The tissue homogenate was centrifuged at 8500 *g* for 10 min and then stored at −70°C until the lipid peroxide assay was performed. The supernatant was collected, and 400 µl ice-cold extraction reagent (ethanol/chloroform, v/v) was added to 250 µl of supernatant in a glass test tube, vortexed for at least 30 s, centrifuged at 3000 *g*, and the upper layer was collected for the SOD assay.

Plasma

Blood was collected in heparinized tubes, and centrifuged at 2500 *g* and 40°C for 5 min.[20] Supernatent plasma was collected and stored at −70°C. When the assay was performed, 400 µl of extraction reagent (ethanol/chloroform) was added to 250 µl of plasma in glass test tubes, vortexed for 30 s, centrifuged at 3000 *g* for 10 min, and the upper layer was collected for the SOD assay.

Serum

Serum samples were separated by centrifugation at 3000 *g* for 5 min and stored at 40°C for examination for lipid peroxides and ANA.

Measurement of lipid peroxidation[21]

Malonaldehyde (MDA) is an end product derived from the breakdown of polyunsaturated fatty acids and related esters. The measurement of such aldehydes provides a conventional index of lipid peroxidation. MDA was measured using the thiobarbituric acid (TBA) assay.

TBA 0.2 mg/dl, 2.5 ml, was added to 0.5 ml serum and/or tissue homogenate (= 100 mg skin biopsy). The mixture was centrifuged at 3500 r.p.m. for 10 min, and the precipitate was washed once with 0.05 M H_2SO_4. Then 2.5 ml 0.05 M H_2SO_4 and 3 ml of TBA 0.2 mg/dl were added, and the mixture was boiled for 30 min. The resulting chromogen was extracted with *n*-butyl alcohol, and the absorbency of the organic phase was determined at a wavelength of 530 nm.

SOD spectrophotometric assay

SOD was assayed spectrophotometrically at a wavelength of 525 nm.[21] The kit was supplied by Oxis International Incorporation Bioxytech SOD-525 (Portland, OR, USA). The biosynTech SOD-525 method is based on the SOD-mediated increase in the rate of autooxidation of tetrahydrotrihydroxybenzofluorene (R1) in an aqueous alkaline solution to yield a chromophore with maximum absorbance at 525 nm. A kinetic measurement of the 525-nm absorbance change is performed after the addition of R1. SOD activity is determined from the ratio of the autooxidation rates in the presence (V_s) and absence (V_c) of SOD. The V_s/V_c ratio as a function of SOD activity is independent of the type of SOD being measured. One SOD 525 activity unit is defined as the activity that doubles the autooxidation rate of the central blank ($V_s/V_c = 2$).

Statistical methods

To compare the different groups we used the ANOVA test for multiple groups and Student's *t*-test for two groups. The relations between the different variables in this study were studied by the Pearson correlation method and by step-wise regression analysis.

Results

Clinical profiles of all the patients are shown in Table 1.

Systemic sclerosis group

Systemic sclerosis patients showed statistically significant elevations in plasma and lesional and nonlesional skin SOD activity, and in and lesional and nonlesional skin serum lipid peroxide levels, when compared with normal controls (Fig. 1). The lesional skin showed a statistically significant elevation of SOD activity and lipid peroxide levels when compared with the nonlesional skin from the same patients.

No significant differences could be detected in blood, lesional, and nonlesional skin SOD activity or lipid peroxide levels when cases of acrosclerosis were compared with those of progressive systemic sclerosis, or when cases of systemic sclerosis alone were compared with those associated with polymyositis or rheumatoid arthritis.

ANA was positive in 13 cases (81.3%). No statistically significant correlation could be detected between the tissue or blood SOD activity or lipid peroxide levels and the ANA titer.

By using step-wise regression analysis, 75.4% of changes in the occurrence of Raynaud's phenomenon could be explained by changes in the nonlesional lipid peroxide levels [R-SQ (adj) = 0.754], and 49.4% of changes in the occurrence of ulceration of the fingertips could be explained by changes in the lesional lipid peroxide levels [R-SQ (adj) = 0.494], while 76.9% of changes in the occurrence of skin induration could be explained by changes in the lesional skin SOD levels [R-SQ (adj) = 0.769].

Morphea group

Morphea patients showed statistically significant elevations in plasma and lesional and nonlesional skin SOD activity and in serum and lesional and nonlesional skin lipid peroxide levels when compared with normal controls (Fig. 1, Table 2). Lesional skin revealed statistically significant elevations of SOD activity and lipid peroxide levels when compared with nonlesional skin from the same patients.

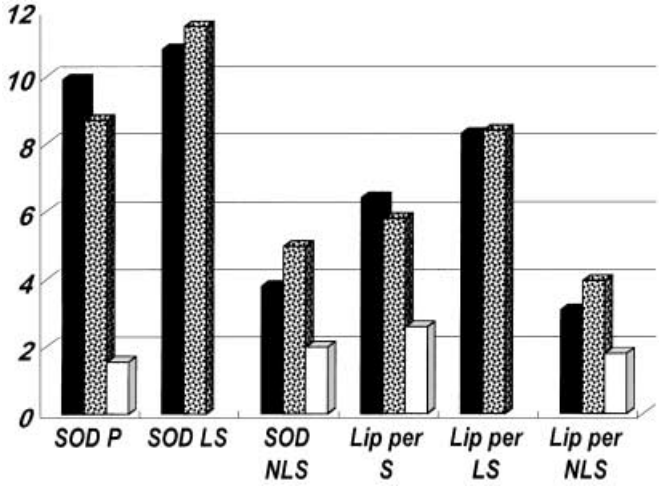

Fig. 1. *Lip per*, lipid peroxide in nmol/ml; *SOD*, superoxide dismutase in U/ml; *P*, plasma; *S*, serum; *LS*, lesional skin; *NLS*, nonlesional skin; *black bars*, systemic sclerosis; *stippled bars*, morphea; *open bars*, controls. *SOD P* (9.9 ± 3.9, 8.7 ± 4.6, 1.6 ± 0.3), *SOD LS* (10.8 ± 4.9, 11.5 ± 5.7), *SOD NLS* (3.8 ± 1.9, 4.9 ± 3.7, 2.0 ± 0.3), *Lip per S* (6.4 ± 2.9, 5.8 ± 2.3, 2.6 ± 0.3), *Lip per LS* (8.3 ± 3.1, 8.4 ± 3.4), and *Lip per NLS* (3.1 ± 1.5, 3.9 ± 1.7, 1.8 ± 0.9) in patients with systemic sclerosis or morphea and in controls, respectively. When either disease was compared with controls, the difference in both *SOD* and lipid peroxide level was statistically significant in nonlesional skin ($P < 0.01$) and in serum, plasma, and lesional skin ($P < 0.001$)

Table 1. Clinical profiles of patients

	Age (years)	Sex	Diagnosis	Overlapping autoimmune disease	Duration (years)	Course	Ind	Dist	Type	R	U	D	ANA HEP 2		
													+/−	Pat	Titer 1/
1	45	F	Systemic sclerosis	–	12	ST	1	Acr	Acr	+	+	+	+	H	160
2	39	F	Systemic sclerosis	Polymyositis	5	PR	1	Acr	Acr	+	−	+	+	SP	320
3	29	F	Systemic sclerosis	–	0.5	PR	3	Acr	Acr	+	+	−	+	SP	160
4	37	F	Systemic sclerosis	–	0.5	PR	3	Acr	Acr	+	−	+	+	N	100
5	15	F	Systemic sclerosis	–	5	PR	2	Acr	Acr	+	+	+		−	
6	24	F	Systemic sclerosis	–	12	ST	1	Acr	Acr	+	+	−	+	SP	160
7	54	M	Systemic sclerosis	–	1	PR	1	Acr	Acr	−	+	−		−	
8	26	M	Systemic sclerosis	–	2	PR	3	Tr&Acr	Pss	+	−	+	+	SP	80
9	21	F	Systemic sclerosis	–	5	ST	2	Acr	Acr	+	+	+	+	H	80
10	50	F	Systemic sclerosis	–	7	ST	1	Acr	Acr	+	+	−	+	H	160
11	37	F	Systemic sclerosis	–	15	ST	1	Acr	Acr	+	+	+	+	SP	80
12	48	F	Systemic sclerosis	Rheumatoid A	0.3	PR	1	Acr	Acr	−	−	−	+	H	320
13	30	F	Systemic sclerosis	Rheumatoid A	20	ST	3	Acr	Acr	+	−	+	+	H	160
14	38	F	Systemic sclerosis	–	10	ST	2	Acr	Acr	+	−	+		−	
15	18	F	Systemic sclerosis	–	7	PR	2	Acr	Acr	+	−	−	+	SP	60
16	30	F	Systemic sclerosis	Polymyositis	0.7	PR	3	Tr&Acr	Pss	+	−	+	+	SP	100
17	38	F	Morphea	–	1.5	ST	2	Tr	Single	−	−	−	+	SP	60
18	38	F	Morphea	–	1.5	PR	3	Limb	Linear	−	−	−	+	SP	80
19	60	M	Morphea	–	2	PR	2	Tr&limb	Diss	−	−	−		−	
20	14	F	Morphea	–	1	PR	3	Face	Single	−	−	−	+	H	60
21	38	M	Morphea	–	2	PR	3	Tr&limb	Diss	−	−	−	+	H	160
22	28	F	Morphea	–	0.25	PR	2	Tr&limb	Diss	−	−	−		−	
23	10	F	Morphea	–	2	ST	1	Limb	Linear	−	−	−	+	SP	120
24	56	F	Morphea	–	1.5	Reg	1	Tr	Single	−	−	−		−	
25	6	M	Morphea	–	2	PR	2	Tr&limb	Diss	−	−	+	+	H	60
26	20	F	Morphea	–	2	ST	1	Face	Linear	−	−	−	+	H&SP	80
27	15	F	Morphea	–	1.5	ST	3	Limb	Linear	−	−	−		−	
28	50	F	Morphea	–	0.3	PR	3	Limb	Linear	−	−	−		−	
29	32	F	Morphea	–	2	ST	1	Tr&limb	Diss	−	−	−		−	
30	38	F	Morphea	–	1	PR	2	Limb	Linear	−	−	−		−	
31	56	F	Morphea	–	1	PR	2	Tr&limb	Gen	−	−	+		−	
32	12	F	Morphea	–	3	PR	1	Tr&limb	Diss	−	−	−		−	
33	39	M	Morphea	–	0.2	PR	3	Limb	Single	−	−	−		−	
34	12	F	Morphea	–	0.3	Reg	0	Limb	Diss	−	−	−		−	
35	27	F	Morphea	–	0.7	PR	3	Limb	Linear	−	−	−		−	
36	12	F	Morphea	–	2	ST	1	Limb	Diss	−	−	−		−	

PR, progressive; ST, stationary; Reg, regressive; Acr, acrosclerosis; Pss, progressive systemic sclerosis; SP, speckled; H, homogenous; N, nuclear; Ind, induration; R, Raynaud's; U, ulceration of the fingertips; D, dysphagia; ANA HEP 2, antinuclear antibodies on HEP 2 cells; Pat, pattern; Dist, distribution; Tr, trunk; Diss, disseminated; Gen, generalized

Table 2. Comparison of the SOD activity and lipid peroxide levels in different groups of morphea with each other and with normal controls

	Single plaque	Linear	Diss & Gen	Controls
SOD in plasma	6.34 ± 2.07 AB	9.97 ± 1.47 A $P < 0.001$*	8.87 ± 1.77 A $P < 0.01$*	1.52 ± 0.116 B
SOD in lesional skin	8.39 ± 8.17 A $P < 0.05$*	11.14 ± 0.64 A $P < 0.001$*	13.11 ± 2.13 A $P < 0.001$*	1.92 ± 0.112 B
SOD in nonlesional skin	3.21 ± 1.01 AB	3.77 ± 1.34 AB	6.72 ± 1.27 A $P < 0.01$*	1.92 ± 0.112 B
Lipid peroxide in serum	4.68 ± 0.26 A $P < 0.01$*	6.94 ± 1.05 A $P < 0.01$*	5.38 ± 0.71 A $P < 0.01$*	2.45 ± 0.14 B
Lipid peroxide in lesional skin	6.33 ± 1.05 A $P < 0.05$*	8.89 ± 1.22 A $P < 0.001$*	8.99 ± 1.29 A $P < 0.001$*	1.5 ± 0.21 B
Lipid peroxide in nonlesional skin	4.28 ± 0.28 A $P < 0.01$*	3.9 ± 0.72 A $P < 0.05$*	4.13 ± 0.61 A $P < 0.01$*	1.5 ± 0.21 B

* Difference is statistically significant

Lipid peroxide, lipid peroxide in nmol/ml serum or tissue homogenate; SOD, superoxide dismutase in SOD 525 U/ml plasma or tissue homogenate

Mean values with the same letter code are not significantly different

ANA was positive in 7 cases (35%) and negative in 13 cases (65%). No statistically significant correlation could be detected between the tissue or blood SOD activity or lipid peroxides and the ANA titer.

When the three groups of morphea patients (single-plaque, linear, and disseminated) were compared with each other, no significant differences could be detected in blood or tissue SOD or lipid peroxide (Table 2).

When each of these groups was compared with the normal controls, it was found (Table 2) that lesional skin SOD activity was higher than that in normal controls in all groups. This difference was statistically significant in the single-plaque group, and highly significant in the disseminated and linear morphea groups. The nonlesional skin SOD activity was significantly higher than that in normal controls only in the group with disseminated morphea. The plasma SOD activity was found to be significantly higher than that in controls in the disseminated and linear morphea groups. The lipid peroxide levels in the serum, and in lesional and nonlesional skin were found to be significantly higher in each group than that in normal controls.

By using step-wise regression analysis, 76.6% of the changes in skin induration could be explained by changes in levels of lesional lipid peroxide (R-SQ (adj) = 0.766), while 66.1% of the changes in skin atrophy could be explained by changes in plasma SOD levels (R-SQ (adj) = 0.661).

Findings in both groups

When the blood, and lesional and nonlesional skin in cases of morphea and systemic sclerosis were compared with each other, no significant differences could be detected (Fig. 1).

SOD activity levels were found to be elevated in the lesional skin of cases with a progressive disease course when compared with those of cases with stationary or regressive disease courses both in the group with morphea (12.02 ± 5.7 U/ml vs. 10.63 ± 5.3 U/ml, respectively, with P = NS) and in the group with systemic sclerosis (11.85 ± 4.9 U/ml vs. 9.54 ± 4.3 U/ml, respectively, with P = NS).

Lipid peroxide levels were found to be elevated in the lesional skin of cases with a progressive disease course when compared with those of cases with stationary or regressive disease courses both in cases with morphea (10.1 ± 2.7 nmol/ml vs. 6.03 ± 2.5 nmol/ml, respectively, with $P < 0.01$) and in those with systemic sclerosis (8.7 ± 3.2 nmol/ml vs. 7.9 ± 2.8 nmol/ml, respectively, with P = NS).

Discussion

The free radical release process appears to play an important role in the pathophysiology of systemic sclerosis[22] as well as in scleroderma due to exogenous factors.[11,12]

Owing to the known pathological and electron microscopic similarities of skin changes in morphea and systemic sclerosis, as well as the items of etiopathogenesis that they have in common,[15] we studied the role of the free radical release process in the pathogenesis of morphea and of systemic sclerosis.

The study included 16 cases of systemic sclerosis and 20 cases of morphea. In the systemic sclerosis cases there were significant elevations of plasma SOD activity and serum lipid peroxide when compared with normal controls. This was consistent with the findings of Morita et al.,[10] who explained this as a reflection of the increased superoxide activity in such cases. The significant elevation of SOD activity and lipid peroxide levels in the lesional skin compared with that in the normal controls as well as that in the normal skin of the same patients probably reflects the importance of the free radical release process and the resulting tissue damage in the development of local tissue changes. This might be supported by the improvement in skin hardening which is found on the topical application of SOD cream in cases of systemic sclerosis.[23] The significant elevation of SOD activity and the lipid peroxide levels in the nonlesional skin of patients when compared with that of controls, together with higher plasma SOD and serum lipid peroxide levels, could reflect a state of increased oxidative activity in these cases. This may be due to excess free radical release,[5] which may transfer and accumulate in healthy skin, or to an increased susceptibility to oxidation.[7–9]

Using step-wise regression analysis revealed that the changes in lesional skin lipid peroxides levels could explain 49.4% of the changes in the occurrence of ulceration of the finger tips, and changes in nonlesional skin lipid peroxidase levels could explain 75.4% of the changes in Raynaud's phenomenon in patients with systemic sclerosis. This may suggest a role of free-radical-mediated tissue damage in the ischemic changes in scleroderma. It has been suggested that ischemia stimulates the release of IL-1. IL-1 enhances oxygen-centered free radicals and stimulates the elaboration of plasminogen activator-1 inhibitor and type-1 collagen in cardiac microvascular endothelial cells, thus leading to the persistence of microvascular thrombi and thus the persistence of tissue ischemia.[24] This could also be the case in systemic sclerosis and the peripheral blood vessels, where ischemia and free radical release may each lead to the other in a vicious circle. This role of free radicals release in vascular changes is suggested in systemic sclerosis, but not in localized scleroderma.

Oxygen free radicals have been found to impair wound healing in ischemic skin,[25] which may be related to the persistence of fingertip ulceration in systemic sclerosis. A positive relation between plasma SOD and the incidence of Raynaud's phenomenon was detected by Morita et al. in 1996.[10] Studying cases of morphea revealed the same finding as for cases of systemic sclerosis. A significant elevation of plasma SOD, serum lipid peroxides, and nonlesional skin SOD and lipid peroxide compared with normal controls reflects increased free-radical release and radical-mediated tissue damage in cases of morphea. The increase in lesional SOD activity and lipid peroxide levels compared with those in nonlesional skin from the same patients and the skin of controls suggests an important role of free-radical release and radical-mediated tissue damage in the pathogenesis of local tissue changes in cases of morphea.

The significant elevation of tissue SOD activity in all morphea groups (single linear and disseminated) compared with that of controls stresses the importance of the role of the free-radical release process in the pathogenesis of localized tissue changes in all types of morphea. The significant elevation of tissue SOD activity in nonlesional skin in cases of disseminated morphea may indicate that skin lesions may occur at any site.

When the morphea group as a whole was compared with the systemic sclerosis group no significant differences were detected in blood, and lesional and nonlesional skin SOD activity or lipid peroxide levels. This indicates that the free-radical release process is involved in the pathogenesis of morphea as well as in that of systemic sclerosis. There were no significant differences in SOD levels in serum between localized scleroderma and systemic sclerosis, and there seems to be little relationship between susceptibility to oxidation and the extent of skin involvement.[8]

It has been found that oxidative stress is often associated with fibrogenesis in different tissues of the body. Lipid peroxidation and certain lipid peroxidation products induce genetic overexpression of fibrogenic cytokines such as transforming growth factor beta (TGF-B) and platelet-derived growth factor,[26] which are suggested to be involved in the pathogenesis of scleroderma. Free-radical-mediated damage to collagen interferes with the normal process of collagen remodeling, i.e., it may interfere with the quality of the newly formed collagen.[27]

By using step-wise regression analysis in both morphea and systemic sclerosis groups, changes in lesional skin SOD activity and lipid peroxide levels could be correlated with the changes in skin induration. It was found that progressive cases have higher lesional skin levels of SOD activity and lipid peroxides than cases with stationary or regressive courses, suggesting that the free-radical-release process and radical-mediated tissue damage are probably involved in the pathogenesis of skin changes in both morphea and systemic sclerosis. This is consistent with the results of Morita et al.,[10] who found a significant correlation between the degree of sclerosis of the skin and the plasma SOD activity.

No statistically significant correlation could be detected between the blood and tissue levels of SOD or lipid peroxides and ANA positivity or titer in cases of morphea or systemic sclerosis. The same finding was reported by Morita et al.,[10] who found no correlation between ANA and Scl 70 titers and serum SOD activity in cases of systemic sclerosis. This would suggest the absence of a direct relationship between the process of autoantibody production and the release of free radicals.

We may therefore conclude that the free-radical-release process is important in the pathogenesis of morphea as it is in systemic sclerosis, where it appears to be involved in the development of skin induration. Free-radical-mediated tissue damage is also suggested to play an important role in the pathogenesis of vascular changes in systemic sclerosis.

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