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Activin, a Grape Seed-derived Proanthocyanidin Extract, Reduces Plasma Levels of Oxidative Stress and Adhesion Molecules (ICAM-1, VCAM-1 and E-selectin) in Systemic Sclerosis

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This study evaluated whether a new generation antioxidant Activin derived from the grape seed proanthocyanidins, could reduce the induction of the adhesion molecules as a result of inflammatory response in the plasma of systemic sclerosis (SSc) patients. SSc patients were divided into two groups: one group was treated with Activin, a grape seed-derived proanthocyanidins, while the other group served as control. Patients were given Activin 100 mg/day orally for one month after which the blood samples were withdrawn from both groups of the patients. Blood was also taken from normal human volunteers. Plasma was obtained in fasting state between 8 to 9 A.M. from two groups of SSc patients and controls. Soluble adhesion molecules including ICAM-1, VCAM-1, E-selectin and P-selectin as well as malonaldehyde, a marker for oxidative stress, were measured. The results of our study demonstrated up-regulation of these soluble adhesion molecules except for P-selectin, in the plasma of the SSc patients compared to those obtained from human volunteers. Activin significantly attenuated the increased expression of these adhesion molecules. In addition, there was a significant increase in the amount of malondialdehyde formation in the plasma of the SSc patients, which was also attenuated by Activin. The results of this study demonstrated that Activin could reduce the inflammatory response and the oxidative stress developed in SSc patients.

Keywords: Activin; Grape-seed proanthocyanidins; Systemic sclerosis; Adhesion molecules; ICAM-1; VCAM-1; E-Selectin; Oxidative stress; Reactive oxygen species; Rheumatoid arthritis

INTRODUCTION

Systemic sclerosis (SSc) affects different physiologic systems, especially the microvasculature.[1] The endothelium is one of the main targets of the disease leading to endothelial injury and apoptosis. The derangement of the endothelium leads to a dysregulation of the vascular tone control, clinically known as Raynaud's phenomenon. This phenomenon induces continuous episodes of reperfusion injury in the circulation of patients with SSc that generates elevated levels of reactive oxygen species (ROS). In SSc, ROS and its products have been shown to be significantly elevated in the circulation[2–4] and it has been hypothesized that the endothelium lining the vessels have an insufficient capacity to deal with this excessive oxidative stress.[5] ROS are now recognized to play a crucial role in the pathophysiology of SSc and constitute today a therapeutic target for different drug option.[6]

In SSc, evidence exists that the circulating levels of soluble adhesion molecules, E-selectin, VCAM and ICAM, are significantly increased.[7–9] The vascular endothelium interacts with circulating leukocytes in cooperation with these adhesion molecules, thus allowing the migration and accumulation of these

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cells in the perivascular space and in the connective tissue.

Grape seed proanthocyanidin is a new generation antioxidant, which not only removes excess amount ROS generated in the disease process, but it also functions as a signaling molecule. Recent studies indicated that grape seed proanthocyanidin ameliorated myocardial ischemic reperfusion injury by reducing ROS in the myocardium.[10–13] This antioxidant exerted its cardioprotective properties by decreasing tissue necrosis and apoptosis through the potentiation of an anti-death signal by reducing pro-apoptotic transcription factors and genes, JNK-1 and c-fos.[13] This study was undertaken to verify, in an open pilot study, if this novel natural antioxidant could be used as potential therapeutic agent to lower the oxidative stress and to interfere with the increase of adhesion molecules in SSc.

PATIENTS

Out of 202 SSc patients admitted in the Department of Medicine of the Section of Rheumatology of the University of Florence, experimental subjects were randomly selected, and the patients were classified according to LeRoy *et al.* (limited SSc and diffuse SSc).[14] Informed consent was obtained from each patient or human volunteer before withdrawing blood samples. Blood was drawn at 9 A.M. from antecubital vein in a fasting state in patients and controls. Smoking subjects were excluded and patients underwent a wash out of 15 days from any antioxidant drug. Only proton pump inhibitors and calcium antagonists were admitted during the study.

MATERIALS AND METHODS

Activin

Activin is a commercially available IH636 grape seed proanthocyanidin extract containing standardized water–ethanol extract from California red grape seeds. This extract mainly contains novel oligomeric proanthocyanidins and a small amount of monomeric flavonoids. High-pressure liquid chromatography (HPLC) analyses in conjunction with gas chromatography-mass spectrometry (GCMS) demonstrated that Activin contains approximately 75–80% oligomeric proanthocyanidins and 3–5% monomeric proanthocyanidins. Figure 1 demonstrates the structures of monomeric, dimeric, trimeric and tetrameric proanthocyanidins. Also, Activin contains 10.8% polysaccharides, 2.1% proteins, 0.5% phytosterols, 2.8% fatty acids, and 5–6% water.

The patient population was randomly divided into two groups. One group was given 100 mg/kg Activin (InterHealth Nutraceuticals, Benicia, CA, USA) per day for 30 days whereas the other group was served as control. Blood samples were also withdrawn from normal human volunteers. The study was performed in double-blinded fashion and the code was revealed only after completion of the experiment.

Assay for Soluble Adhesion Molecules

Plasma was assayed in triplicate for VCAM-1, ICAM-1, E-selectin and P-selectin by enzyme-linked immunosorbent assay. All assays were performed with commercially available kits (R&D Systems Incorporation, Minneapolis, MN) according to the manufacturer's instructions.

Assay for Malondialdehyde

Malondialdehyde (MDA) was assayed as described previously, to monitor the development of oxidative stress.[15] In short, plasma samples were put in 2 ml of a solution containing 20% trichloroacetic acid, 5.3 mM sodium bisulfite, kept on ice for 10 min, centrifuged at 3,000*g* for 10 min, and then supernatants were collected, derivatized with 2,4-dinitrophenyl-hydrazine (DNPH), and extracted with pentane. Aliquots of 25 μl in acetonitrile were injected onto a Beckman Ultrasphere C_{18} (3 μm) column. The products were eluted isocratically with a mobile phase containing acetonitrile–water–acetic acid (40:60:0.1, v/v/v) and measured at three different wavelengths (307, 325 and 356 nm) using a Waters M-490 multichannel UV detector. The peak for malondialdehyde was identified by co-chromatography with DNPH derivative of the authentic standard, peak addition, UV pattern of absorption at the three wavelengths, and by GC-MS.

Statistical Analysis

The Statistical analysis was performed by a two-way analysis of variance for repeated measures followed by a multiple comparison Scheffe's test to determine differences between groups. The paired Student's *t*-test was used for within-group comparisons with baseline values. The values were expressed as mean ± SEM. The results were considered significant if p was less than 0.05.

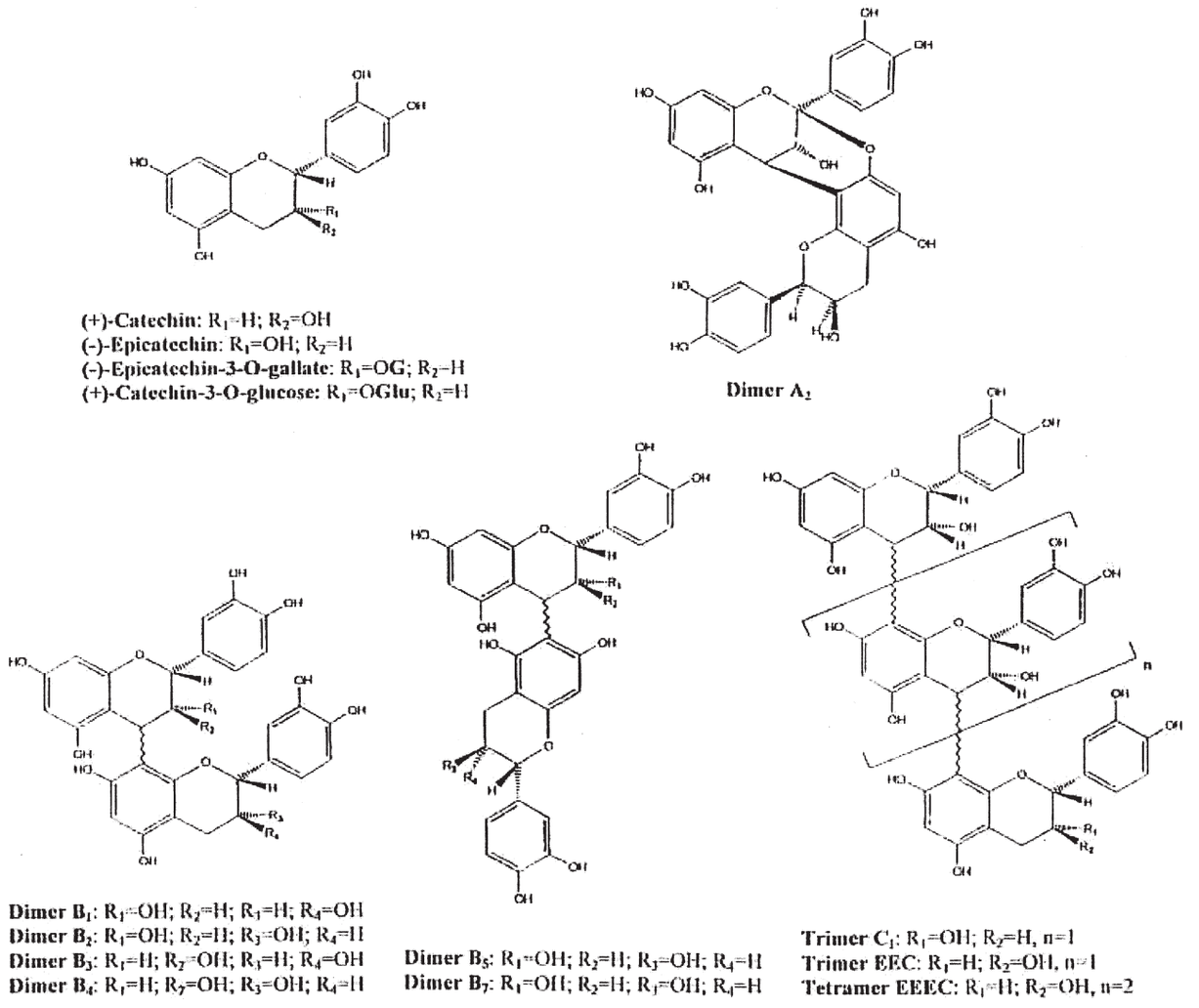

FIGURE 1 Chemical structure of Activin which is a mixture of proanthocyanidin monomers, dimers, trimers and tetramers.

RESULTS

Effects of SSc on the Plasma Levels of Adhesion Molecules

We first measured plasma levels of ICAM-1, VCAM-1, E-selectin and P-selectin from the normal human volunteers and SSc patients. ICAM-1, VCAM-1, and E-selectin, but not P-selectin, in plasma of SSc patients were increased significantly as compared to those found in the plasma of normal volunteers (Fig. 2).

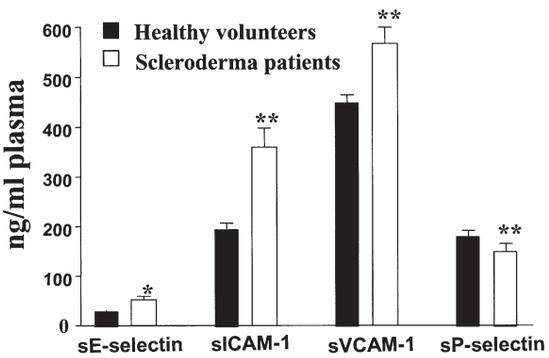

FIGURE 2 Quantitation of soluble adhesion molecules sE-Selectin, sICAM-1, sVCAM-1, and P-Selectin in the serum of healthy volunteers and scleroderma patients. Results are expressed as Mean ± SEM. Significant differences among the two experimental groups were calculated using Mann–Whitney test for unpaired groups, $**P < 0.01$, $***P < 0.001$.

Effect of Activin on the Expression of Soluble Adhesion Molecules

After one month of treatment, in the patients with Activin a significant reduction of the circulating levels of ICAM-1, VCAM-1 and E-selectin were found when compared to those found for SSc

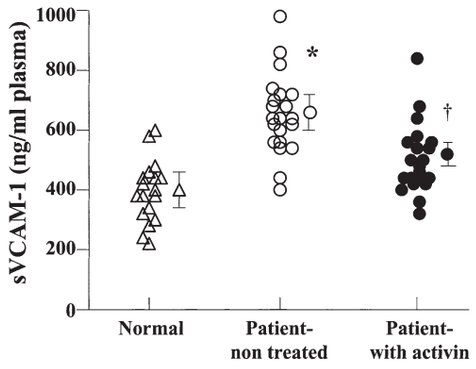

FIGURE 3 Effects of Activin on sVCAM-1 from the rheumatoid arthritis patients. A group of the patients were given Activin, 100 mg/kg/day for 30 days while the other group served as Activin control. Blood samples were withdrawn from the normal human volunteers which served as control. At the end of 30 days, sVCAM-1 expression was determined in the plasma samples from the two groups of rheumatoid arthritis patients and one group of normal volunteers as described in the "Methods Section". Results are expressed as Means ± SEM of six hearts per group.

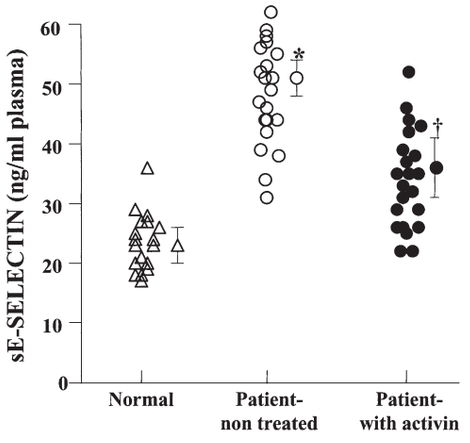

FIGURE 5 Effects of Activin on sE-Selectin from the rheumatoid arthritis patients. A group of the patients were given Activin, 100 mg/kg/day for 30 days while the other group served as Activin control. Blood samples were withdrawn from the normal human volunteers which served as control. At the end of 30 days, sE-Selectin expression was determined in the plasma samples from the two groups of rheumatoid arthritis patients and one group of normal volunteers as described in the "Methods Section". Results are expressed as Means ± SEM of six hearts per group.

patients not treated with Activin (Figs. 3–6). No side effects were detected during the treatment. Patients not treated with Activin (placebo only) did not show a significant reduction of the circulating levels of adhesion molecules.

Effects of Activin on MDA Formation

After a month, Activin reduced the MDA level in the plasma of the patients suggesting that Activin had a peculiar *in vivo* activity as an antioxidant reducing the oxidative stress developed by reperfusion injury in SSc (Fig. 7). Patients treated with placebo did not show any significant reduction of MDA.

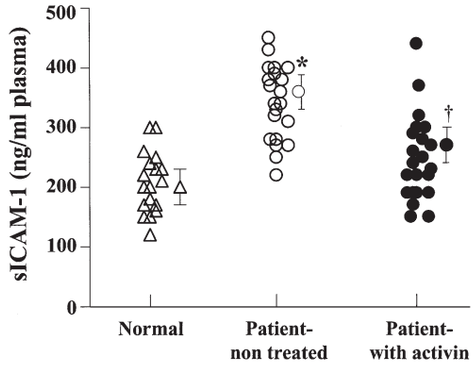

FIGURE 4 Effects of Activin on sICAM-1 from the rheumatoid arthritis patients. A group of the patients were given Activin, 100 mg/kg/day for 30 days while the other group served as Activin control. Blood samples were withdrawn from the normal human volunteers which served as control. At the end of 30 days, sICAM-1 expression was determined in the plasma samples from the two groups of rheumatoid arthritis patients and one group of normal volunteers as described in the "Methods Section". Results are expressed as Means ± SEM of six hearts per group.

DISCUSSION

The results of the present study clearly show that feeding of grape seed proanthocyanidins to the SSc patients for one month reduced the amount of soluble adhesion molecules, ICAM-1, VCAM-1 and E-selectin in the plasma samples compared to placebo patients. Proanthocyanidins are known to function as antioxidants, and is supported by the evidence that MDA content in the plasma of the proanthocyanidin-fed patients was also lower compared to those without proanthocyanidins. These

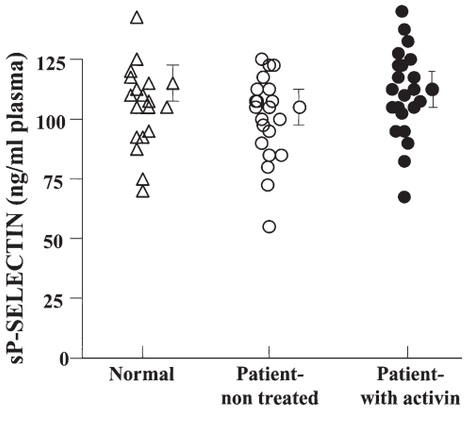

FIGURE 6 Effects of Activin on sP-Selectin from the rheumatoid arthritis patients. A group of the patients were given Activin, 100 mg/kg/day for 30 days while the other group served as Activin control. Blood samples were withdrawn from the normal human volunteers which served as control. At the end of 30 days, sP-selectin expression was determined in the plasma samples from the two groups of rheumatoid arthritis patients and one group of normal volunteers as described in the "Methods Section". Results are expressed as Means ± SEM of six hearts per group.

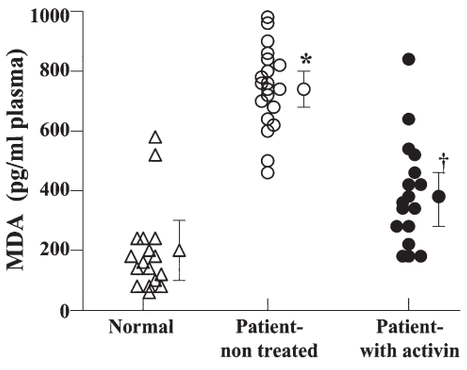

FIGURE 7 Effects of Activin on the MDA content of the plasma from the rheumatoid arthritis patients. A group of the patients were given Activin, 100 mg/kg/day for 30 days while the other group served as Activin control. Blood samples were withdrawn from the normal human volunteers which served as control. At the end of 30 days, MDA content was determined in the plasma samples from the two groups of rheumatoid arthritis patients and one group of normal volunteers as described in the "Methods Section". Results are expressed as Means ± SEM of six hearts per group.

results may suggest that Activin is able to reduce oxidative stress as well as to down-regulate the mechanisms leading to the adhesion and homing of leucocytes in SSc patients.

Substantial evidence exists in the literature indicating a crucial role of ROS in SSc.[4–7] While infiltrating activated leukocytes in the affected organs in SSc (skin, lung, etc.) are one of the major source of ROS (belch), oxygen free radicals are generated mainly with the hypoxic/reoxygenation injury following continuous attacks of Raynaud's phenomenon in SSc.

The adhesion of leukocytes to endothelial cells requires the expression of leukocyte-specific adhesion proteins on the surface of the vascular endothelium.[16] Upon up-regulation, these adhesion molecules are capable of attachment to activated polymorphonuclear neutrophils, allowing transendothelial migration and cytotoxic damage. We measured four soluble adhesion molecules constitutively expressed on the vascular endothelium. The results of the present study confirm that there is a significant up-regulation of three of these adhesion molecules including ICAM-1, VCAM-1 and E-selectin in the serum of SSc patients. Activin, a grape seed derived proanthocyanidin extract, decreased the amount of adhesion molecules. Elevations of these adhesion molecules definitively indicate activation or damage to the vascular endothelium.[17] Although the biologic significance of these circulating soluble adhesion molecules is unclear, it has been reported that sE-selectin can up-regulate neutrophils integrin function, thereby acting as a physiologic proadhesive effector.[16] In addition, targeted reduction of adhesion molecule expression has been used to reduce ischemia/reperfusion injury. It has been demonstrated that a monoclonal antibody against ICAM-1 significantly attenuated the increase in polymorphonuclear neutrophils adherence to ischemic/reperfused coronary endothelium.[17] Regardless of the specific biologic roles for these soluble adhesion molecules, their up-regulation signifies an increased inflammatory endothelial response and their down-regulation means relieve from the inflammatory response.

In concert, the amount of oxidative stress developed in the system was reduced as evidenced by the decrease in the amount of MDA formation. MDA is a presumptive marker for lipid peroxidation resulting from lipid-free radical interaction. The results of this study demonstrated significant reduction in the amount of MDA formation in the plasma of the rheumatoid patients after Activin treatment. ROS play a significant role in the pathogenesis of a variety of disease processes including rheumatoid arthritis.[18] However, the mechanism by which ROS can increase the expression of the adhesion molecules is not clear.

Several other groups have used antioxidants in SSc. For example, Denton *et al.* treated SSc patients with probucol and obtained an excellent result in reducing the number of attacks of Raynaud's phenomenon.[19] Our group has treated SSc patients with infusion of *N*-acteyl cysteine and found a reduction of attacks of Raynaud's phenomenon and the number of ulcers.[20] Other micronutrients failed to show any effect on SSc.[6]

In this study, we used a novel second generation antioxidant derived from the grape seeds. The antioxidant effectiveness of these polyphenolic compounds have been found to be several times more potent than vitamin E in their free radical scavenging properties as also in their ability to prevent LDL oxidation.[21] Flavonoids are primarily derived from the skins, seeds and stems of grapes while anthocyanins come predominantly from the skins. Proanthocyanidins comprise a group of polyphenolic bioflavonoids ubiquitously found in the lignified portions of grape clusters, especially in the seeds. Recently, grape seed proanthocyanidins were found to possess cardioprotective abilities by functioning as *in vivo* antioxidant and by virtue of their ability to directly counteract oxidative and nitrosative stress.[10–13,22,23] We have also demonstrated that Activin can significantly inhibit cancer chemotherapeutic drugs-induced toxicity by modulating apoptotic regulatory genes bcl_2 and p53.[24] Our study clearly demonstrated that grape seed proanthocyanidins inhibited the *in vivo* production of the reactive oxygen species in the ischemic reperfused myocardium.[10,13]

Apart from functioning as an intracellular antioxidant, the grape seed-derived proanthocyanidin

extract can also provide cardioprotection by antagonizing the death signal mediated through the ischemia/reperfusion-induced activation of JNK-1 and c-Jun.[12] Previous studies have demonstrated that JNKs are activated during the reperfusion of ischemic myocardium.[25] It is tempting to speculate that the oligomeric proanthocyanidins inhibited the induction of the adhesion molecules by down-regulating the expression of JNK-1 and c-Jun. Indeed, a recent study demonstrated that quercetin, a naturally occurring pentahydroxylavone, could decrease the ICAM-1 expression by decreasing the transcription factor AP-1 through the JNK-pathway.[26] In the recent past, we evaluated the effects of Activin on the expression of TNFα-induced ICAM-1 and VCAM-1 expression in primary human umbilical endothelial cells (HUVEC). Activin at low concentrations (1–5 μg/ml), down-regulated TNFα-induced VCAM-1 expression but not ICAM-1 expression in HUVEC. Such regulation of inducible VCAM-1 by Activin was also observed at the mRNA expression level. We conducted a cell–cell co-culture assay to verify whether the inhibitory effect of Activin on the expression of VCAM-1 was also effective in down-regulating actual endothelial cell/leukocyte interaction. Activin treatment significantly decreased TNFα-induced adherence of Jurkat T-cells to HUVEC.[27] Another study demonstrated that TNFα up-regulates ICAM-1 expression through the activation of the JNK/SAPK transduction pathway mediated by the p55 receptor.[28] Another related study showed that pyrrolidine dithiocarbamate (PDTC) exerted proinflammatory action by sustained JNK-c-JUN activation which translated into long-lasting ICAM production.[29] Thus, it seems likely that Activin decreased the induction of the adhesion molecules by down-regulating the expression of JNK-1, which is activated during inflammatory response.

In summary, the results of our study demonstrate that a second generation antioxidant, Activin, derived from grape seeds, reduces the expression of the soluble adhesion molecules, ICAM-1, VCAM-1 and E-selectin and also reduces the amount of oxidative stress in the plasma of SSc patients. Future studies will determine whether this extract will be effective in protecting the endothelium from injury and apoptosis as well as in controlling the dysfunction of vascular tone.

Acknowledgements

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References

[1] Cerinic, M.M., Kahaleh, B.M. and LeRoy, E.C. (1995) "The vascular involvement in systemic sclerosis", In: Furst, D. and Clements, P., eds, *Systemic Sclerosis* (Lea and Febiger, Philadelphia, PA).

[2] Simonini, G., Pignone, A., Generini, S., Falcini, F., Cerinic, M.M., Gabriele, S., Alberto, P., Sergio, G., Fernanda, F. and Marco, M.C. (2000) "Emerging potentials for an antioxidant therapy as a new approach to the treatment of systemic sclerosis", *Toxicology* **155**, 1–15.

[3] Cerinic, M.M., Generini, S. and Pignone, A. (1997) "New approaches to Raynaud's phenomenon", *Curr. Opin. Rheumatol.* **9**, 544–556.

[4] Simonini, G., Cerinic, M.M., Generini, S., Zoppi, M., Anichini, M., Cesaretti, A., Pignone, A., Falcini, F., Lotti, T. and Cagnoni, M. (1999) "Oxidative stress in systemic sclerosis", *Mol. Cell. Biochem.* **196**, 85–91.

[5] Cerinic, M.M., Generini, S., Pignone, A. and Cagnoni, M. (1998) "From Raynaud's phenomenon to systemic sclerosis: lack or exhaustion of adaptation?", *Adv. Organ Biol.* **6**, 241–253.

[6] Herrick, A.L. and Cerinic, M.M. (2001) "The emerging problem of oxidative stress and the role of antioxidants in systemic sclerosis", *Clin. Exp. Rheumatol.* **19**, 4–8.

[7] Roberts, S., Kosanke, S., Terrence, D.S., Jankelow, D., Duran, C.M. and Cunningham, M.W. (2001) "Pathogenic mechanisms in rheumatic carditis: focus on valvular endothelium", *J. Infect. Dis.* **183**, 507–511.

[8] Chang, D.M., Kuo, S.Y., Lai, J.H. and Chang, M.L. (1999) "Effects of anti-rheumatic herbal medicines on cellular adhesion molecules", *Ann. Rheum. Dis.* **58**, 366–371.

[9] Della Rossa, A., Valentini, G., Bombardieri, S., Bencivelli, W., Silman, A.J., D'Angelo, S., Cerinic, M.M., Belch, J.F., Black, C.M., Becvar, R., Bruhlman, P., Cozzi, F., Czirjak, L., Drosos, A.A., Dziankowska, B., Ferri, C., Gabrielli, A., Giacomelli, R., Hayem, G., Inanc, M., McHugh, N.J., Nielsen, H., Scorza, R., Tirri, E., van den Hoogen, F.H. and Vlachoyiannopoulos, P.G. (2001) "European multicentre study to define disease activity criteria for systemic sclerosis. I. Clinical and epidemiological features of 290 patients from 19 centres", *Ann. Rheum. Dis.* **60**, 585–591.

[10] Sato, M., Maulik, G., Ray, P.S., Bagchi, D. and Das, D.K. (1999) "Cardioprotective effects of grape seed proanthocyanidin against ischemic reperfusion injury", *J. Mol. Cell. Cardiol.* **31**, 1289–1297.

[11] Sato, M., Ray, P.S., Maulik, G., Maulik, N., Engelman, R.M., Bertelli, A.A.E. and Das, D.K. (2000) "Myocardial protection with red wine extract", *J. Cardiovasc. Pharmacol.* **35**, 263–268.

[12] Sato, M., Bagchi, D., Tosaki, A. and Das, D.K. (2001) "Grape seed proanthocyanidin reduces cardiomyocyte apoptosis by inhibiting ischemia/reperfusion-induced activation of JNK-1 and c-JUN", *Free Radic. Biol. Med.* **31**, 729–737.

[13] Pataki, T., Bak, I., Kovacs, P., Bagchi, D., Das, D.K. and Tosaki, A. (2002) "Grape seed proanthocyanidins reduced ischemia/reperfusion-induced injury in isolated rat hearts", *Am. J. Clin. Nutr.*, in press.

[14] LeRoy, E.C. and Medsger, Jr., T.A. (2001) "Criteria for the classification of early systemic sclerosis", *J. Rheumatol.* **28**, 1573–1576.

[15] Cordis, G.A., Bagchi, D., Maulik, N. and Das, D.K. (1994) "High-performance liquid chromatographic method for the simultaneous detection of malonaldehyde, acetaldehyde, formaldehyde, acetone and proprionaldehyde to monitor the oxidative stress in heart", *J. Chromatogr. A* **661**, 181–191.

[16] Godin, C., Caprani, A., Dufaux, J. and Flaud, P. (1993) "Interactions between neutrophils and endothelial cells", *J. Cell Sci.* **106**, 441–452.

[17] Gearing, A.J.H. and Newman, W. (1993) "Circulating adhesion molecules in disease", *Immunol. Today* **14**, 506–512.

[18] Grootveld, M., Silwood, C.J., Lynch, E.J., Patel, I.Y. and Blake, D.R. (1999) "The role of N-acetylcysteine in protecting synovial fluid biomolecules against radiolytically-mediated oxidative damage: a high field proton NMR study", *Free Radic. Res.* **30**, 351–359.

[19] Denton, C.P., Bunce, T.D., Darado, M.B., Roberts, Z., Wilson, H., Howell, K., Bruckdorfer, K.R. and Black, C.M. (1999)

"Probucol improves symptoms and reduces lipoprotein oxidation susceptibility in patients with Raynaud's phenomenon", *Rheumatology (Oxford)* **38**, 309–315.
[20] Herrick, A.L., Rieley, F., Schofield, D., Hollis, S., Braganza, J.M. and Jayson, M.I. (1994) "Micronutrient antioxidant status in patients with primary Raynaud's phenomenon and systemic sclerosis", *J. Rheumatol.* **21**, 1477–1483.
[21] Das, D.K., Sato, M., Ray, P.S., Maulik, G., Engelman, R.M., Bertelli, A.A.E. and Bertelli, A. (1999) "Cardioprotection by red wine: role of polyphenolic antioxidants", *Drugs Exp. Clin. Res.* **25**, 115–120.
[22] Bagchi, D., Bagchi, M., Stohs, S.J., Das, D.K., Ray, S.D., Kuszymski, C.A., Joshi, S.S. and Preuss, H.G. (2000) "Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention", *Toxicology* **148**, 187–197.
[23] Roychowdhury, S., Wolf, G., Keilhoff, G., Bagchi, D. and Horn, T. (2001) "Grape seed proanthocyanidin extract (GSPE) protects glia against nitrosative/oxidative stress", *Nitric Oxide* **5**, 137–149.
[24] Joshi, S., Kuszynski, C.A., Benner, E.J., Bagchi, M. and Bagchi, D. (1999) "Amelioration of cytotoxic effects of idarubicin and 4-hydroxyperoxycyclophosphamide on Chang liver cells by a novel grape seed proanthocyanidin extract", *Antioxid. Redox Signal.* **1**, 563–570.
[25] Sato, M., Cordis, G.A., Maulik, N. and Das, D.K. (2000) "SAPKs regulation of ischemic preconditioning", *Am. J. Physiol.-Heart Circ. Physiol.* **279**, H901–H907.
[26] Kobuchi, H., Roy, S., Sen, C.K. and Packer, L. (1999) "Quercetin inhibits inducible ICAM-1 expression in human endothelial cells through the JNK pathway", *Am. J. Physiol.-Lung Cell. Mol. Physiol.* **277**, C403–C411.
[27] Sen, C.K. and Bagchi, D. (2001) "Regulation of inducible adhesion molecule expression in human endothelial cells by grape seed proanthocyanidin extract", *Mol. Cell. Biochem.* **216**, 1–7.
[28] DeCasaris, P., Starace, D., Starace, G., Filippini, A., Stefanini, M. and Ziparo, E. (1999) "Activation of Jun *N*-terminal kinase/stress-activated protein kinase pathway by TNFα leads to intracellular adhesion molecule-1 expression", *J. Biol. Chem.* **274**, 28978–28982.
[29] Liao, H.L., Zhu, Y., Verna, L. and Stemerman, M.B. (2000) "Selective activation of endothelial cells by the antioxidant pyrrolidine dithicarbamate of C-jun *N*-terminal kinase and AP-1 activation", *Endothelium* **7**, 121–133.