Chapter 10
Role of Oxidative Stress and Reactive Oxygen Radicals in the Pathogenesis of Systemic Sclerosis

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Abstract Systemic sclerosis (SSc) is an autoimmune connective tissue disease which causes progressive fibrosis of skin and numerous internal organs. Although the etiology of SSc is unknown and the detailed mechanisms responsible for the fibrotic process have not been elucidated, there is strong evidence to support the concept that oxidative stress mediated by an excessive generation of oxidative free radicals plays a crucial role. Elevated levels of markers of oxidative stress and reduced levels of antioxidants have been found in SSc patients, and the most commonly studied animal models of SSc are induced by chemical agents that generate oxidative stress. In this chapter, the available evidence for the participation of oxidative stress in SSc pathogenesis will be reviewed emphasizing the link between free radicals and the process of fibrosis and the potentially beneficial effects of antioxidant treatment for the disease.

Abbreviations

ADA	Adenosine deaminase
Col I	Type I collagen
ECM	Extracellular matrix
ECS	Extract from fruits of *Capparis spinosa* L.
EGCG	Epigallocatechin-3-gallate
ERK1/2	Extracellular signal-regulated kinase 1/2
ET-1	Endothelin 1
IPF	Idiopathic pulmonary fibrosis
NAC	*N*-acetylcysteine

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NOX	NADPH oxidase
PDGFR	Platelet-derived growth factor receptor
PG	Prostaglandin
((PHTE)(2)NQ)	2,3-bis(phenyltellanyl)naphthoquinone
PTP1B	Protein tyrosine phosphatase 1B
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SSc	Systemic sclerosis
TGF-β1	Transforming growth factor-β1

10.1 Introduction

Systemic sclerosis (scleroderma, SSc) is an autoimmune inflammatory disease of unknown etiology characterized by pronounced fibroproliferative alterations in the microvasculature, cellular and humoral immunity abnormalities, and an often progressive severe fibrotic process affecting the skin and many internal organs [1–3].

The pathogenesis of SSc is complex, and despite numerous studies that have examined several aspects of its intricate picture, the exact mechanisms involved are not well understood. However, it is apparent that its most severe clinical and pathologic manifestations are the result of a fibrotic process characterized by the excessive and often progressive deposition of collagen and other connective tissue macromolecules in skin, heart, lungs, and many other internal organs. The cells responsible for this process are activated fibroblasts also known as myofibroblasts. These cells display an upregulated expression of genes encoding various collagens and other extracellular matrix (ECM) proteins in affected organs. This is the most important difference that distinguishes normal fibroblasts that promote normal wound healing from SSc fibroblasts which display exaggerated and uncontrolled collagen and ECM production resulting in pathologic organ fibrosis [3, 4]. Regardless of the etiologic event, the accumulation of activated fibroblasts or myofibroblasts in affected tissues and the persistence of their elevated biosynthetic functions are responsible for the extent and rate of progression of the fibrotic process which is the main determinant of SSc's clinical course, response to therapy, prognosis, and mortality. Although the mechanisms involved in the initiation and progression of the remarkable fibrotic process in SSc remain largely unknown, oxidative stress has been implicated in SSc pathogenesis. Numerous studies have demonstrated that oxidative stress participates in some aspects of each of the three crucial pathological processes of SSc, i.e., the fibroproliferative vasculopathy, the cutaneous and visceral fibrosis, and the cellular and humoral immunity abnormalities. This review will focus on the studies examining the role of oxidative stress in the pathogenesis of the fibrotic process in SSc.

10.2 Evidence of Oxidative Stress in SSc

Following the early work of Murrell [5] suggesting that oxidative stress may play a role in SSc pathogenesis, a number of studies have confirmed these initial observations and have explored the mechanisms involved [6, 7]. Owing to the crucial role of the fibrotic process in SSc clinical manifestations, most of these investigations have focused on the involvement of oxidative stress in the pathogenesis of tissue fibrosis. It has been shown that oxidative stress in SSc is the result of an imbalance between the production of oxidative stress producing systems and their antagonist antioxidant mechanisms. Indeed, reduction of endogenous antioxidants and a parallel overproduction of reactive oxygen species (ROS) have been documented in SSc, although increased generation or overproduction of ROS appears to be the main mechanism whereas ROS inactivation is believed to play a less important role. Several studies demonstrated decreased levels of antioxidants in serum of SSc patients [8, 9] although other studies have not confirmed these observations [10]. Furthermore, numerous others have shown increased ROS production [11–14] providing strong evidence to the important contribution of oxidative stress in the pathogenesis of the disease.

Numerous oxidative stress-related products have been detected in various biological fluids from SSc patients supporting the concept of increased oxidative stress in SSc, including increased urinary 8-oxodG levels [15] or presence of increased levels of isoprostanes in SSc serum [16, 17]. Isoprostanes such as F2-isoprostanes are markers of lipid peroxidation (for review, see ref. [18]) produced in vivo in humans by the non-cyclooxygenase, free radical-catalyzed, peroxidation of arachidonic acid and have been shown to be a reliable measure of lipid peroxidation in vivo. Increased levels of urinary F2IP-M, a bioactive prostaglandin (PG)F_2-like compound, produced by this pathway was also found to be elevated in urine from SSc patients [19]. Another isoprostane, 8-isoprostane, was described as a biomarker of oxidative stress in interstitial lung diseases [20]. The level of oxidative stress has also been shown to be enhanced in patients with interstitial lung diseases such as fibrosing alveolitis associated with SSc as reflected by increased concentrations of 8-epi-$PGF_{2\alpha}$ in bronchoalveolar lavage fluid [21]. Other markers of oxidative stress in SSc include increased serum levels of N(epsilon)-(hexanoyl)lysine [22] and elevated serum levels of heat shock protein 70 [23].

Clastogenic activity measured in plasma has also been evaluated as a biomarker of oxidative stress in SSc [24]. Inosine triphosphate, the deamination product of ATP, is one of the clastogenic and superoxide generating components of clastogenic plasma factors. Clastogenic activity is found not only in the plasma but also in the supernatants of fibroblast cultures obtained from SSc patients. This activity was correlated with an increase of adenosine deaminase (ADA), an ubiquitous enzyme involved in purine metabolism. ADA catalyzes the irreversible deamination of adenosine or deoxyadenosine leading to the formation of inosine triphosphate and inosine diphosphate, which are abnormal nucleotides with clastogenic and superoxide stimulating properties.

10.3 Reactive Oxygen Species

ROS are a group of oxygen-derived molecules characterized by high chemical reactivity. ROS play both a deleterious and a beneficial dual role. Under physiological conditions, ROS perform important functions in cellular signaling pathways and host defense, but in pathological states, they can induce oxidative stress causing damage to proteins, lipids, and DNA, as well as activating various redox-sensitive cell signaling pathways [25]. ROS include the free superoxide ($^{\bullet}O_2^-$) and hydroxyl ($^{\bullet}OH$) radicals and the non-radical species, hydrogen peroxide (H_2O_2), hypochlorous acid (HClO), and peroxynitrite ($ONOO^-$). Although several enzyme systems are capable of producing ROS including the mitochondria electron transport chain, lipoxygenases, cyclooxygenases, xanthine oxidase, cytochrome P450, uncoupled eNOS, and peroxisomes, numerous studies have shown that NADPH oxidases are the primary enzymes responsible for inducible ROS formation. NADPH oxidase generation of ROS has been documented in many systems and organs including the vascular system [26–28], lungs [29], kidneys [30], and neurologic system [31].

10.3.1 ROS in the Pathogenesis of SSc

Recent studies have implicated excessive oxidative stress and the generation of deleterious ROS in the pathogenesis of SSc [7, 14, 32]. Although ROS are produced by normal fibroblasts and are essential for numerous intracellular functions including fibroblast proliferation, there is evidence that SSc dermal fibroblasts produce increased ROS levels [14] and that elevated ROS may be involved in the increased collagen expression in these cells. Also, monocytes from SSc patients spontaneously release increased amounts of superoxide anion in vitro [11]. These studies demonstrated that increased ROS production by either untreated or phorbol 12-myristate 13-acetate-stimulated monocytes was mediated through the activation of NADPH. These results are in agreement with previous studies that suggested that NADPH oxidases are the most important mediators of ROS production by fibroblasts. Other studies in support of the potential role of ROS in the pathogenesis of fibrotic and vascular lesions in SSc included the demonstration that sera from patients with SSc induce high production of ROS by endothelial cells and fibroblasts in vitro and that the levels of induction are correlated with the severity of clinical involvement [13]. It is of substantial relevance that ROS participate in important signaling cascades and pathways in numerous types of cells including those that are activated in SSc such as fibroblasts, myofibroblasts, endothelial cells, or pericytes. It has also been recently shown that ROS may cause the oxidation of DNA-topoisomerase-1 and that through this mechanism, ROS can also mediate the induction of immunologic intolerance to this nuclear antigen [33]. Anti-DNA-topoisomerase-1 antibody, also known as Scl-70 antibody, the prototypic marker autoantibody in diffuse SSc, is found in serum of patients with diffuse cutaneous SSc but is not detected in

patients with limited cutaneous SSc or other connective tissue disorders. In these studies which employed a novel SSc animal model, it was demonstrated that HOCl or •OH induced high levels of oxidized DNA-topoisomerase-1 and that this protein modification increased DNA-topoisomerase-1 antigenicity, a required initial step for the development of the autoantibody.

A recent study described a novel mechanism by which ROS may promote a profibrotic phenotype in SSc fibroblasts [34]. This mechanism involves the oxidative inactivation of protein tyrosine phosphatase 1B (PTP1B) leading to pronounced platelet-derived growth factor receptor (PDGFR) activation. This study also provided a novel molecular mechanism by which therapy with *N*-acetylcysteine (NAC), a thiol antioxidant, may be beneficial for SSc by acting on ROS and PTP1B. ROS production and expression of type I collagen (Col I), a fibrotic marker gene, were significantly higher in SSc, and these alterations were accompanied by significantly lower amounts of free thiols compared to normal fibroblasts. In this study, it was also shown that following PDGF stimulation, PDGFR and extracellular signal-regulated kinase 1/2 (ERK1/2) were phosphorylated to a greater extent, whereas the ability to produce PTP1B was substantially diminished in SSc fibroblasts. Furthermore, PTP1B activity was significantly reduced in SSc fibroblasts, most likely as a result of increased cysteine oxidation caused by higher levels of ROS. Confirmation of the important role of PTP1B on the regulation of the fibrotic process was obtained from studies showing that decreased PTP1B expression in normal fibroblasts led to increased Col I. Treatment of SSc cells with NAC restored the low PTP1B activity, improved the profile of p-PDGFR, decreased the amounts of tyrosine-phosphorylated proteins and Col I, and scavenged ROS in SSc fibroblasts [34]. These studies collectively provide strong support for the participation of ROS not only in the development of exaggerated fibrotic responses in SSc but also in the generation of disease-specific autoantibodies and other immunologic alterations characteristic of the disease.

10.4 NADPH Oxidases

The NADPH oxidase (NOX) family of enzymes, which catalyze the reduction of O_2 to form ROS, are likely to have crucial roles in normal cellular physiology as evidenced by the remarkable increase in the number of NOX enzymes during eukaryotic evolution with seven distinct NOX isoforms identified in mammals [35–37]. The role of NOX enzymes in a variety of human disorders is currently the focus of intense investigation, and there is strong experimental evidence to support their participation in the fibrotic process. Transforming growth factor-β1 (TGF-β1), the most important pro-fibrogenic growth factor, has been shown to be a potent inducer of ROS generation in myofibroblasts, the cells responsible for exaggerated ECM production in SSc. Furthermore, the NOX4 isoform, one of the seven members of the NOX family, has been identified as a source of ROS generation in cardiac and lung myofibroblasts, and it has also been implicated in the process of TGF-β-induced differentiation of

quiescent cardiac fibroblasts to myofibroblasts [38, 39]. NOX4 is upregulated in lungs of patients with idiopathic pulmonary fibrosis (IPF) and in lung mesenchymal cells, and NOX4-dependent generation of H_2O_2 is required for TGF-β1-induced myofibroblast differentiation and increased ECM production [39]. Furthermore, abrogation of NOX4 has been shown to inhibit fibrogenesis in two different murine models of lung injury [39]. Generation of H_2O_2 by lung myofibroblast NOX4 mediates additional fibrogenic effects by inducing epithelial cell apoptosis [40]. Therefore, numerous studies have provided strong supporting evidence for a role of NOX4 in normal myofibroblast tissue repair functions and in abnormal, exaggerated fibrogenesis. Thus, there is a very cogent and strong rationale for therapeutic targeting of NOX4 in abrogating lung fibrosis and other fibrotic disorders such as SSc. The development of small molecule inhibitors and/or other strategies targeting NOX4 offers substantial promise for the treatment of currently incurable fibrotic disorders, such as IPF and SSc.

10.4.1 Regulation of NOX Activity

The mechanisms involved in the regulation of NOX activity are quite complex, and it is likely that they vary depending on a specific cellular and functional context. There are four general mechanisms of NOX regulation: (1) regulation of expression of NOX genes or of the genes encoding regulatory subunits of the corresponding NOX isoenzymes including modulation of mRNA translation or stability, (2) regulation of NOX protein stability and turnover, (3) regulation of enzymatic activity, and (4) feedback or other endogenous inhibitory mechanisms. Among all the NOX enzymes, it appears that NOX4 is the most likely member of this extended family of ROS-generating enzymes to play a role in some of the complex molecular pathways involved in the SSc fibrotic process. It is important to emphasize that, in contrast with all other NOX enzymes, NOX4 does not require other protein subunits for its activity, and, therefore, enzyme activity is dependent on the expression level of its corresponding gene. Given the important functions of NOX4 in a variety of physiological processes and in the pathogenesis of numerous diseases, there has been intense interest in unveiling the intimate mechanisms of its regulation.

Although the exact mechanisms involved in the regulation of NOX4 levels in normal cells are becoming unveiled, the possible alterations responsible for the constitutive elevation in NOX4 levels and activity in SSc cells are not known. Multiple growth factors and related polypeptides which contribute to the activation of NADPH oxidases have been shown to participate in SSc pathogenesis. Among these are the following: (a) TGF-β, a well known cytokine implicated in the fibrotic process [4, 41] which stimulates transmembrane serine/threonine kinases activating NOX4 [38]; (b) PDGF which also plays an important role in the pathogenesis of SSc [42] has been extensively studied following the initial demonstration that this growth factor was capable of potent stimulation of fibroblast proliferation as well as of causing increased production of ECM by mesenchymal

cells and activation of NOX1 and NOX4 [43, 44]; (c) angiotensin II, which has recently been implicated in fibrosis through its ability to induce oxidative stress [45, 46]; and (d) endothelin-1, another potent profibrotic factor able to cause elevated ROS production; indeed, it has been found that endothelin-1 (ET-1) induces NADPH oxidase in human endothelial cells [47].

Recent studies have shown that antibodies to PDGFR also induce the production of ROS [48, 49]. This is a novel mechanism that provides a cogent link between the recently identified PDGF antibodies in the sera of SSc patients [49] and the pathogenesis of the fibrotic process. Studies of the mechanisms of PDGF-induced stimulation of ROS production have shown that PDGF increased ROS, and through this effect, it may regulate Ras protein levels via ERK1/2. These studies found that PDGF induced posttranscriptionally Ha-Ras by stimulating ROS and ERK1/2. Activation of ERK1/2 and high ROS levels were shown to stabilize Ha-Ras protein, by inhibiting its proteasomal degradation. The increased levels of ROS induced by PDGF resulted in increased Ha-Ras and active ERK1/2 and subsequently in stimulation of collagen synthesis, DNA damage, and accelerated senescence.

Thus, stabilization of Ras protein by ROS and ERK1/2 in primary fibroblasts induced by PDGF amplifies the response of the cells to growth factors, and this mechanism may represent a critical factor in SSc onset and progression [50].

10.5 Animal Models of Oxidative Stress-Induced Tissue Fibrosis

Animal models are extremely valuable to examine and validate various hypotheses about the pathogenesis of human diseases and, most importantly, to develop and test potentially effective therapeutic interventions before they can be applied to humans. Several animal models have been developed to examine various aspects of SSc pathogenesis or of the fibrotic process associated with SSc and other fibrotic disorders [51–55].

Although most of the animal models for SSc do not reproduce the entire spectrum of pathogenic mechanisms proposed for the disease, there are several induced animal models that reproduce some of the most relevant pathophysiologic alterations of the disease. Among these, the most commonly and extensively utilized animal model is the one induced by intratracheal or subcutaneous injections of bleomycin, a free radical generator [56–60]. Bleomycin-induced skin fibrosis mimics early changes in SSc, and it has been suggested that one of the most likely mechanisms involved is the increased production of ROS which then damage the surrounding cells, such as endothelial cells.

Another recently described animal model of SSc is induced by repeated and prolonged (daily for 6 weeks) intradermal injections of HOCl, which generate hydroxyl radicals and result in the development of severe fibrosis in skin and lungs with characteristics resembling those of diffuse cutaneous SSc in humans [33]. The fibrosis persists for at least 10 weeks following the last administration

of HOCl. The skin of these mice contained large numbers of myofibroblasts with increased rate of proliferation and increased production of collagen and α-smooth muscle actin, the molecular marker of their transdifferentiation into activated myofibroblasts. The serum of these mice was found to harbor increased levels of IgG and IgM and anti-DNA-topoisomerase-1 antibodies. In a variation of this animal model, the authors also described that treatment of mice with agents capable of generating peroxynitrite anions induced changes consistent with limited cutaneous SSc, including the absence of lung involvement and the production of anti-centromere binding protein-B antibodies, which are serologic markers considered to be highly specific for the limited form of SSc.

10.6 Antioxidant Systems

Mammalian cells contain numerous and quite efficient systems capable of counteracting the potent and pleiotropic effects of oxidative stress. These include antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutases (SOD), which are found in mitochondria, cytosol, plasma membranes, and the extracellular space. SOD, which converts superoxide radicals to hydrogen peroxide, functions as a highly efficient defense mechanism against ROS effects. The important role of SOD in the abrogation of ROS-mediated profibrotic mechanisms has recently been supported by interesting studies describing a novel autoantibody to Cu/Zn SOD present in sera of patients with localized scleroderma [61]. These authors hypothesized that the autoimmune background in localized scleroderma induced anti-Cu/Zn SOD autoantibodies that inhibited SOD activity and thereby contributed to fibrosis by increasing ROS. In other studies, it was found that alterations in SOD3 expression and activity were associated with SSc fibrosis [62]. The increased expression of SOD3 mRNA and enzymatic activity detected in SSc fibroblasts, as compared to control healthy fibroblasts in these studies, may have represented a protective mechanism attempting to balance the elevated ROS effects.

10.7 Antioxidant Treatments

Owing to the importance of the oxidative process in SSc pathogenesis and to the observations that patients with SSc have reduced serum concentrations of the natural antioxidants, ascorbic acid, α-tocopherol, and β-carotene, as well as low values of selenium, a large number of antioxidants have been used with therapeutic purposes [63]. The therapeutic application of various antioxidant agents for SSc has been supported by provocative in vitro results with SSc fibroblasts [34] as well as studies in various SSc animal models. In one of these studies, edaravone, a free radical scavenger, has been used in two different mouse models of SSc and a significant

inhibitory effect on fibrosis was observed [64]. In another recent study, the efficiency of a tellurium-based catalyst, 2,3-bis(phenyltellanyl)naphthoquinone ((PHTE)(2)NQ), in the treatment of HOCl-induced murine model of SSc was demonstrated. (PHTE)(2)NQ efficiency is linked to the selective pro-oxidative and cytotoxic effects of this compound on hyperproliferative fibroblasts [65]. Another putative antioxidant molecule, α-melanocyte-stimulating hormone, suppressed bleomycin-induced oxidative stress, reduced skin fibrosis and collagen content, and increased tissue levels of superoxide dismutase 2 (SOD2) and heme oxygenase-1 in a murine model of SSc and was, therefore, suggested as a potential therapy for SSc [66].

Despite these highly encouraging results from animal models, the clinical observations employing various antioxidant agents, however, have not consistently demonstrated beneficial effects. For example, a short-term treatment with vitamin E in SSc patients failed to decrease the basal rate of lipid peroxidation and did not improve microvascular perfusion following cold exposure in these patients [67]. However, in related studies, Allanore et al. found acute and sustained effects of dihydropyridine-type calcium-channel antagonists on oxidative stress in SSc [68], and nifedipine, one of the most commonly used dihydropyridine-type calcium-channel antagonist, was found to protect against overproduction of superoxide anion by peripheral blood monocytes from SSc patients. Further studies on this topic showed that this beneficial property of nifedipine appeared to be mediated both by calcium-channel cellular action and by the inhibition of protein kinase C activity [12].

Besides its potent effect causing reduction of disulfide bonds in susceptible proteins, the thiol compound NAC exerts a strong antioxidant effect. Therefore, there has been intense interest in the possible beneficial effects of NAC in the treatment of SSc. One of the earliest studies of NAC in SSc was conducted by Furst et al. [69] in 22 SSc patients. The parallel, double-blind, placebo-controlled prospective trial of 1 year in duration failed to show any significant differences in various relevant parameters between the placebo and the NAC-treated patients. A subsequent study by Sambo et al. [70] evaluated the effects of a short-term (5 days) intravenous infusion of NAC in patients with SSc-associated Raynaud's phenomenon. Although there was a reduction in the number of digital ulcers and in the recovery time following cold challenge, it is difficult to interpret the validity of the results because the study was not placebo-controlled. A more extensive study of intravenous NAC therapy was reported by Salsano et al. in 2005 [71]. These authors examined the effects of intravenous infusion of NAC given daily for 5 h for 8 months, during the winter (from October to May) for 2 years. The results showed increased hand perfusion assessed by laser Doppler perfusion imaging. However, owing to the fact that the study was uncontrolled, the beneficial effects reported need to be interpreted cautiously. Three recent studies from the same group of investigators examined the effects of a similar therapeutic regime of intravenous NAC on kidney and liver perfusion in SSc patients showing beneficial effects with an improvement in hepatic perfusion [72] and a reduction in renal artery resistance index [73]. Highly encouraging results were also obtained in a long-term prospective study of 50 patients with a previous history of severe Raynaud's phenomenon with digital ulcers who had

failed to respond to calcium-channel blocker therapy. The patients received NAC intravenously for a duration of 5 h every 14 days for an average of 4.2 ± 2.11 years. In this study, there was a substantial reduction in the numbers of digital ulcers and in the frequency and severity of Raynaud's phenomenon episodes. An assessment of the long term effects of NAC intravenous therapy on various parameters of lung function in the same cohort of SSc patients was recently published [74]. The results indicated that NAC caused stabilization or minimal improvement in pulmonary function studies with stabilization of in the parenchymal abnormalities assessed by high-resolution chest CT scans. Although the results of the later studies are encouraging, the lack of a placebo control group suggests that these conclusions will need to be validated in properly controlled studies.

Numerous recent studies have focused on novel classes of antioxidant compounds with encouraging results. One study showed that the antioxidant epigallocatechin-3-gallate (EGCG) was able to reduce ECM production by dermal fibroblasts from SSc patients in culture, modulating collagen type I and fibronectin gene expression, reducing the fibrotic marker connective tissue growth factor, and inhibiting collagen gel contraction. EGCG was also able to suppress intracellular ROS, ERK1/2 kinase signaling, and nuclear factor-κB activity [75].

It has been suggested that an extract from fruits of *Capparis spinosa* L. (ECS) protects against oxidative stress in SSc dermal fibroblasts. ECS exhibits a notable activity in protecting against oxidative stress and interrupting the ROS-ERK1/2-Ha-Ras signal cascade in SSc fibroblasts, suggesting that it may exert a potential protective effect against the development of skin sclerosis. ECS significantly reduced the production of O_2^-, H_2O_2, and ROS in SSc fibroblasts and minimized the loss of cell viability and apoptosis induced by H_2O_2 in normal and SSc fibroblasts [76].

Iloprost, a stable synthetic analogue of prostacyclin, currently employed in the treatment of SSc vascular features, also possesses strong anti-oxidative properties beside its prostaglandin-like vasodilatory and platelet antiaggregation effects. Indeed, it has been shown that a standard course of iloprost therapy was capable of acutely reducing oxidative stress in SSc patients. This effect appeared to be more consistent in patients with the early phases of SSc and in the limited subset of disease [77, 78]. However, earlier studies on the effects of iloprost in vivo have shown that its strong vasodilator effect did not reduce oxidative status because urinary 8-isoPGF$_{2\alpha}$ did not diminish following its administration to SSc patients [79]; thus, the role of iloprost as an antioxidant and its potential therapeutic benefits in SSc remains to be conclusively determined.

Among other potential antioxidants that may have beneficial effects in the therapy of SSc patients is activin, a grape seed-derived proanthocyanidin extract, which has been found to reduce plasma levels of oxidative stress markers in SSc. Indeed, malondialdehyde, a marker for oxidative stress which was increased in the plasma of the SSc patients, was significantly reduced by the administration of activin [80].

Although the numerous studies discussed above suggesting that various antioxidants may be of benefit for the therapy of SSc patients have strong support from

in vitro and in vivo studies employing experimental animal models, their beneficial effects need to be conclusively demonstrated in rigorously controlled clinical trials in SSc patients.

10.8 Conclusion

There is strong experimental evidence to suggest that oxidative stress contributes to the progression of fibrosis in SSc and that these effects appear to be caused by excessive and unbalanced generation of ROS in affected tissues. Therefore, the potential use of antioxidants in the treatment of SSc has been suggested. However, clinical trials with some of these antioxidant agents have failed to show conclusively documented positive results. Thus, it will be necessary to perform further in vitro studies and well-controlled placebo-matched clinical trials to document conclusively any beneficial effects. Furthermore, it is possible that the use of combined antioxidants with other drugs may result in improved therapy for this disabling and frequently fatal disease.

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