EXTENDED REPORT

The 12/15-lipoxygenase pathway counteracts fibroblast activation and experimental fibrosis

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ABSTRACT

Background Idiopathic and inflammation-dependent fibrotic diseases such as systemic sclerosis (SSc) impose a major burden on modern societies. Understanding endogenous mechanisms, which counteract fibrosis, may yield new therapeutic approaches. Lipoxins are highly potent lipid mediators, which have recently been found to be decreased in SSc.

Objectives To determine the potential role of 12/15-lipoxygenase (12/15-LO), the key enzyme for the synthesis of lipoxins, in fibrosis.

Methods Two mouse models for experimental dermal fibrosis (bleomycin-induced dermal fibrosis and tight-skin 1 mouse model) together with bone marrow transfers were used in wildtype and 12/15-LO^{-/-} mice to elucidate the role of this enzyme during dermal fibrosis. Primary dermal fibroblasts of wildtype and 12/15-LO^{-/-} mice, and 12/15-LO-derived eicosanoids, were used to identify underlying molecular mechanisms

Results In both models, 12/15-LO^{-/-} mice exhibited a significant exacerbation of the fibrotic tissue response. Bone marrow transfer experiments disclosed a predominant role of mesenchymal cell-derived 12/15-LO in these antifibrotic effects. Indeed, 12/15-LO^{-/-} fibroblasts showed an enhanced activation of the mitogen-activated protein-kinase pathway and an increased col 1a2 mRNA expression in response to stimulation with transforming growth factor β (TGF β), whereas 12/15-LO-derived eicosanoids blocked these TGF β -induced effects.

Conclusions These data indicate that 12/15-LO and its metabolites have a prominent antifibrotic role during dermal fibrosis. This opens new opportunities for therapeutic approaches in the treatment of fibrotic diseases.

INTRODUCTION

Systemic sclerosis (SSc) is a rare connective tissue disease with unknown aetiology that affects the skin and a variety of internal organs, including the lungs, the gastrointestinal tract and the heart.¹ Histopathological hallmarks of early stages of SSc are perivascular inflammatory infiltrates and a reduced capillary density. Later stages of the disease are characterised by an excessive accumulation of extracellular matrix (ECM) components.² The resulting fibrosis is a major cause of death in SSc as it often results in dysfunction of the affected organs. The enhanced production of ECM components in patients with SSc is mediated by activated fibroblasts, which produce increased amounts of

ECM.¹ However, the molecular mechanisms of fibroblast activation and potential counter-regulatory mechanisms, which limit the inflammatory reaction and the consecutive ECM accumulation, are incompletely understood.

Eicosanoids, such as prostaglandins, leukotrienes and lipoxins, have been shown to act as key modulators of inflammation and the following phases of resolution and tissue repair.³ During the initial phase of the inflammatory response cyclooxygenase-2 and 5-lipoxygenase (5-LO) catalyse the generation of prostaglandins and leukotrienes. While leukotrienes, primarily exert proinflammatory signals, prostaglandins have been shown to act in a proinflammatory and anti-inflammatory manner, depending on the context. Other arachidonic acid-metabolising enzymes, including human 15-lipoxygenase-1 (15-LO) and its murine orthologue the 12/15-lipoxygenase (12/15-LO), are expressed in the final stage of inflammation. These enzymes have been implicated in the generation of a subclass of lipid mediators, including lipoxin A₄ (LXA₄), which initiate the resolution phase of inflammation and exert potent anti-inflammatory effects.^{3–5} These different classes of eicosanoids have been also implicated in the regulation of postinflammatory tissue repair, which involves regulation of ECM production and fibrotic tissue response.^{6,7} Of particular interest, studies have demonstrated a reduction of lipoxins in the bronchoalveolar lavage of patients with SSc compared with controls,^{8,9} indicating that lipoxins and 12/15-LO may have a role in the pathogenesis of SSc. Nevertheless, the molecular role of lipoxins and 12/15-LO in SSc has not been analysed so far.

In this study, we aimed to elucidate the role of 12/15-LO in dermal fibrosis. Using 12/15-LO-deficient mice, the bleomycin-induced model of skin-fibrosis and bone marrow transplantations, we demonstrate a protective role of this enzyme during inflammation-induced skin fibroses, which correlates with production of LXA₄. 12/15-LO expression in mesenchymal, but not in haematopoietic cells, was required for this protective effect. By crossing 12/15-LO^{-/-} mice into the tight-skin-1 (tsk-1) background, we confirmed a direct antifibrotic and protective role of 12/15-LO during tissue fibrosis in vivo. The following in vitro analysis of 12/15-LO^{-/-} fibroblasts showed an enhanced activation state, including an aberrant activation of the mitogen-activated protein (MAP)-kinase pathway

Figure 1 12/15-Lipoxygenase (12/15-LO) deficiency increases the susceptibility to bleomycin-induced dermal fibrosis. Representative skin sections (stained with haematoxylin and eosin, x100) (A), quantification of dermal thickness (B), of myofibroblast content (C) and of hydroxyproline content (D) after histological analysis of the skin of 12/15-LO^{+/+} and 12/15-LO^{-/-} mice, which were treated with NaCl and bleomycin, respectively. (E) Results of the measurement of the lipoxin A₄ content by competitive ELISA. The levels of NaCl-treated 12/15-LO^{+/+} mice were defined as 1; other results were normalised to this value. Data represent the mean ± SEM. *p < 0.05; **p < 0.01.

and overwhelming production of collagen in the absence of 12/15-LO. Conversely, addition of different 12/15-LO-derived eicosanoids, including LXA₄, reversed this phenotype.

MATERIAL AND METHODS

Bleomycin-induced dermal fibrosis in 12/15-LO-deficient mice

Mice deficient for 12/15-LO (12/15-LO^{-/-}) have been described earlier¹⁰ and were purchased from the Jackson Laboratory (Bar Harbor, Maine, USA). 12/15-LO^{-/-} mice were maintained on a C57Bl/6 background. Matched wildtype C57Bl/6 mice expressing 12/15-LO (12/15-LO^{+/+}) from the same breeding groups were used as controls. Skin fibrosis was induced in 6-week-old mice by local injections of bleomycin for 4 weeks as described.^{11 12} Briefly, 100 µl of bleomycin dissolved in 0.9% sodium chloride (NaCl) at a concentration of 0.5 mg/ml was administered every other day by subcutaneous injections in defined areas of 1 cm² at the upper back. Subcutaneous injections of 100 µl 0.9% NaCl were used as controls. Four different groups, two consisting of 12/15-LO^{-/-} mice and two of 12/15-LO^{+/+} mice, were analysed. One group of 12/15-LO^{-/-} mice and one group of 12/15-LO^{+/+} mice were challenged with bleomycin, whereas the other groups were injected with NaCl. Mice were killed by cervical dislocation after 4 weeks. Each group consisted of six to eight mice.

Bone marrow transplantation

To investigate the relative contribution of fibroblasts and other mesenchymal cells, on the one hand, and bone marrow-derived cells, on the other, to the phenotype of 12/15-LO^{-/-} mice, bone marrow transplantation experiments were performed as described.¹³ Female 12/15-LO^{-/-} and 12/15-LO^{+/+} mice served as donors for bone marrow. For isolation of bone marrow cells, tibial

and femur bones were prepared under sterile conditions. Bone marrow cells were flushed from the bone marrow cavities with phosphate-buffered saline and subsequently filtered through 70 µm nylon meshes (BD Biosciences, Heidelberg, Germany). After lyses of erythrocytes, the isolated bone marrow cells were transplanted without further purification or in vitro expansion. Male 12/15-LO^{-/-} or 12/15-LO^{+/+} mice were transplanted at an age of 4 weeks. Twenty hours before transplantation, recipient 12/15-LO^{-/-} or 12/15-LO^{+/+} mice underwent whole-body irradiation with 11 Gy. For transplantation, 2.0 × 10⁶ bone marrow cells were injected into the tail veins. Two weeks after bone marrow transplantation and after confirmation of a complete reconstitution of the haematopoiesis, mice were challenged with bleomycin for 4 weeks as described above. The different experimental groups consisted of six to nine animals each.

12/15-LO deficiency in tight-skin 1 mice

In addition to the mouse model of bleomycin-induced dermal fibrosis, the *tsk-1* mouse model was used to evaluate the effects of 12/15-LO deficiency on fibrosis. The *tsk-1* phenotype is caused by a dominant mutation of the fibrillin-1 gene. Mice carrying one mutated fibrillin-1 gene (*FBN1*^{*tsk-1/wildtype*}) are characterised by tethered skin with accumulation of collagen in the hypodermis and hypodermal thickening.¹⁴ *Tsk-1* mice were crossed with 12/15-LO^{-/-} mice. The resulting F1 generations were backcrossed with 12/15-LO^{+/+} or 12/15-LO^{-/-} mice. The F2 generations were killed at an age of 10 weeks to analyse the hypodermal thickness and the number of myofibroblasts.

Histological analysis

The dermal thickness was analysed at 100-fold magnification by measuring the distance between the epidermal—dermal junction

Figure 2 Absence of 12/15-lipoxygenase (12/15-LO) in resident mesenchymal cells exacerbates experimental fibrosis. Representative skin sections (stained with haematoxylin and eosin, x100) (A), quantification of dermal thickness (B) and of myofibroblast content (C) after histological analysis of the skin of 12/15-LO^{+/+} and 12/15-LO^{-/-} mice after indicated bone marrow (BM) transplantation and treatment with NaCl and bleomycin, respectively. The levels of bleomycin-treated 12/15-LO^{+/+} mice transplanted with bone marrow (BM) from wildtype mice were defined as 1; other results were normalised to this value. Data represent the mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$.

and the dermal–subcutaneous fat junction at three sites from lesional skin of each mouse as described.^{13 15 16} Collagen fibres were visualised by Masson's trichrome staining (Sigma-Aldrich, Munich, Germany) and analysed at 1000-fold magnification. Images were captured with a Nikon Eclipse 80i microscope (Badhoevedorp, Netherlands) equipped with a DSP 3CCD camera (Sony, Tokyo, Japan).

Detection of myofibroblasts

Myofibroblasts were identified by immunohistochemistry for α -smooth muscle actin as described.^{17 18}

Cell culture

Isolation and cultivation of murine dermal fibroblasts was performed as previously described.¹⁹

Lipoxin A₄ measurement

Competitive ELISA techniques were used to determine levels of LXA₄ (Oxford Biomedical Research Inc), 13-S-HODE and PGE₂ (both kits from assay designs). Tissue samples were homogenised and the respective eicosanoids were isolated according to the protocol provided by the manufacturer using solid-phase columns.

Eicosanoids

12-HETE and 15-HETE (Cayman Chemicals, Ann Arbor, MI, USA) were used at a concentration of 100 nmol/l, 13-HODE (Cayman Chemicals) at a concentration of 5 μ mol/l and LXA₄ (Sigma, Munich, Germany) at 500 nmol/l.

Quantitative real-time PCR

Reverse transcription (RT)-PCR was performed as previously described.²⁰ RNA was isolated using TRIZOL reagent (Invitrogen,

Darmstadt, Germany). Total RNA (900 ng) was reverse transcribed with human leukaemia virus reverse transcriptase using the Gene Amp RNA PCR kit (Applied Biosystems, Foster City, California, USA) and oligo(dT) primers. mRNA levels were normalised to β -actin expression. The following primer sequences were used:

col1a1 fwd	GAA GCA CGT CTG GTT TGG A
col1a1 rev	ACT CGA ACG GGA ATC CAT C
col1a2 fwd	CCA ACA AGC ATG TCT GGT TAG GA
col1a2 rev	TCA AAC TGG CTG CCA CCA T

Western Blot analysis

Cells and tissue were homogenised and lysed in Laemmli buffer. Protein concentration was determined using an RC/DC protein quantification kit (Biorad, Hercules, California, USA). Proteins were separated by electrophoresis in 12% sodium dodecyl sulphate-polyacrylamide gels. Proteins were blotted onto polyvinylidene difluoride membranes and, after blocking with 5% dry milk/0.1% Tween 20, incubated with primary and secondary peroxidase-conjugated antibodies and detected by chemiluminescence.

Statistics

Data are expressed as mean $\pm$ SE of the mean. The Mann–Whitney U test was used for statistical analyses. A p value < 0.05 was considered statistically significant.

RESULTS

12/15-LO deficiency increases the susceptibility to bleomycin-induced dermal fibrosis

To investigate the role of 12/15-LO in dermal fibrosis we used the bleomycin model of skin fibrosis. Skin architecture and dermal thickness did not differ between 12/15-LO^{-/-} mice and

Figure 3 12/15-Lipoxygenase (12/15-LO) deficiency augments fibrosis in tight-skin-1 (tsk-1) mice. Representative skin sections (stained with haematoxylin and eosin, x40) (A), quantification of dermal thickness (B) and of myofibroblast content (C) after histological analysis of the skin of 12/15-LO^{+/+}/FBN1^{wildtype/wildtype}, 12/15-LO^{-/-}/FBN1^{wildtype/wildtype}, 12/15-LO^{+/+}/FBN1^{tsk-1/wildtype} and 12/15-LO^{-/-}/FBN1^{tsk-1/wildtype}, which were treated with NaCl and bleomycin, respectively. FBN, fibrillin-1 gene. The levels of 12/15-LO^{+/+}/FBN1^{wildtype/wildtype} mice were defined as 1; other results were normalized to this value. Data represent the mean±SEM. *p<0.05; **p<0.01.

12/15-LO^{+/+} mice injected with NaCl, suggesting that the skin phenotype is not generally altered in 12/15-LO^{-/-} mice (figure 1A,B). Upon injection of bleomycin, however, a significantly increased fibrotic response was seen in 12/15-LO^{-/-} mice. The dermal thickness increased by 90±2% in 12/15-LO^{-/-} mice compared with 42±4% in 12/15-LO^{+/+} mice (p=0.001) (figure 1A,B).

Consistent with the increased dermal thickness, the number of myofibroblasts upon challenge with bleomycin was significantly higher in 12/15-LO^{-/-} mice than in 12/15-LO^{+/+} mice (p=0.022) (figure 1C). Moreover, the increase in hydroxyproline content upon challenge with bleomycin was significantly more pronounced in 12/15-LO^{-/-} mice than in 12/15-LO^{+/+} littermates (figure 1D). These data show that deficiency of 12/15-LO increases the susceptibility to bleomycin-induced fibrosis and indicates a protective and antifibrotic role of 12/15-LO during bleomycin-induced fibrosis.

To further determine underlying mechanisms, we performed a measurement of the LXA₄ content in the skin of 12/15-LO^{+/+} and 12/15-LO^{-/-} mice after NaCl and bleomycin challenge, respectively. LXA₄ represents a major 12/15-LO metabolite and has been shown to exert both anti-inflammatory and antifibrotic effects in vitro.^{21,22} While we did not observe a difference in dermal LXA₄ levels after injection of NaCl, LXA₄ levels significantly increased after injection of bleomycin. Consistent with a central role of 12/15-LO in the generation of LXA₄, 12/15-LO^{-/-} mice displayed significantly reduced amount of LXA₄ (figure 1E).

Absence of 12/15-LO in resident mesenchymal cells exacerbates experimental fibrosis

The mouse model of bleomycin-induced fibrosis is an inflammation-dependent model, in which bleomycin induces the influx

of leucocytes into lesional skin with secondary activation of fibroblasts by leucocyte-derived profibrotic cytokines.²³ Thus, aggravated fibrosis in this model might be linked to an increased inflammatory response in the form of an enhanced activation of cells of haematopoietic origin, such as leucocytes. Alternatively, an altered responsiveness of mesenchymal fibroblasts may directly cause an enhanced fibrotic response. To determine whether the protective effects of 12/15-LO during bleomycin-induced skin fibrosis are linked to 12/15-LO expression in cells of haematopoietic or of mesenchymal origin, we performed a series of bone marrow transplantations. The resulting chimeric mice were challenged with bleomycin. Bleomycin challenge of 12/15-LO^{+/+} mice reconstituted with bone marrow from either 12/15-LO^{-/-} or 12/15-LO^{+/+} resulted in a comparable increase in dermal thickness with increases of 54±10% and 54±11%, respectively, compared with NaCl-treated mice (figure 2A,B).

In contrast, 12/15-LO^{-/-} mice showed increased susceptibility to bleomycin-induced fibrosis regardless of whether or not they were reconstituted with bone marrow cells derived from 12/15-LO^{+/+} mice or from 12/15-LO^{-/-} mice. Challenge of 12/15-LO^{-/-} mice reconstituted with 12/15-LO^{+/+} bone marrow with bleomycin resulted in a 90±14% increase in dermal thickness. This increase did not differ from that seen in 12/15-LO^{-/-} mice with 12/15-LO^{-/-} bone marrow (p=0.33), but was significantly more pronounced than in 12/15-LO^{+/+} mice with 12/15-LO^{+/+} bone marrow and in 12/15-LO^{+/+} mice reconstituted with 12/15-LO^{-/-} bone marrow (p=0.002 and p=0.001, respectively) (figure 2A,B). The increased differentiation of resting fibroblasts into metabolically active myofibroblasts in 12/15-LO^{-/-} mice was also seen in 12/15-LO^{-/-} mice transplanted with 12/15-LO^{+/+} bone marrow, whereas the myofibroblast

Figure 4 12/15-Lipoxygenase (12/15-LO)-deficient fibroblasts show an enhanced response to transforming growth factor β (TGF β). (A) Primary dermal fibroblasts of 12/15-LO^{+/+} and 12/15-LO^{-/-} mice were stimulated with TGF β (10 ng/ml) for the indicated times and phosphorylation of the indicated mitogen-activated protein (MAP)-kinase pathways was analysed by Western Blot. (B) After stimulation of fibroblasts with TGF β (10 ng/ml) for 8 h, mRNA expression of col 1a2 was determined by real-time PCR.

counts in 12/15-LO^{+/+} mice transplanted with 12/15-LO^{-/-} bone marrow did not differ from those of 12/15-LO^{+/+} mice (figure 2C). Together, these data demonstrate that 12/15-LO expression in resident mesenchymal cells, rather than in haematopoietic cells, accounts for the protective effect of 12/15-LO during experimental fibrosis.

12/15-LO deficiency augments fibrosis in *tsk-1* mice

To confirm a role of the 12/15-LO pathway in another model of skin fibrosis, we used the *tsk-1* model. In contrast to the bleomycin-induced model, the *tsk-1* model is an inflammation-independent model, in which fibrosis is mainly driven by endogenous activation of fibroblasts.²³ Mice lacking 12/15-LO were therefore crossed with *tsk-1* mice to obtain *tsk-1* mice lacking both alleles for 12/15-LO (12/15-LO^{-/-}/FBN1^{tsk1/wildtype}). 12/15-LO^{-/-}/*tsk-1* mice were born in the expected Mendelian ratios and appeared

completely normal at birth. Skin fibrosis was more pronounced in 12/15-LO^{-/-}/FBN1^{tsk1/wildtype} mice than in their 12/15-LO^{+/+}/FBN1^{tsk1/wildtype} littermates. Here, we observed a significantly increased hypodermal thickening and increased myofibroblast differentiation (figure 3A–C). No differences in hypodermal thickness were seen between 12/15-LO^{-/-}/FBN1^{wildtype/wildtype} mice and 12/15-LO^{+/+}/FBN1^{wildtype/wildtype}. These data confirm our previous results on the protective, antifibrotic role of 12/15-LO in bleomycin-induced dermal fibrosis and suggest that the 12/15-LO pathway directly affects the response of fibroblasts, including the regulation of myofibroblast differentiation and ECM accumulation.

12/15-LO-deficient fibroblasts show an enhanced response to TGF β

To further elucidate underlying molecular mechanisms, we focused on the role of 12/15-LO during fibroblast activation. Therefore, fibroblasts derived from 12/15-LO^{+/+} and 12/15-LO^{-/-} mice were stimulated with TGF β . MAP-kinase activation was monitored by Western Blot analysis, which showed increased activation of all three MAP-kinase pathways in the absence of 12/15-LO (figure 4A). Moreover, the analysis of the downstream collagen gene expression in the respective fibroblasts by quantitative real-time PCR showed significant differences. Fibroblasts isolated from 12/15-LO-deficient animals displayed increased expression of col 1a2 mRNA as compared with 12/15-LO^{+/+} control fibroblasts (figure 4B).

12/15-LO-derived eicosanoids counteract TGF β -induced activation of fibroblasts

Next, we determined the effects of different 12/15-LO-derived eicosanoids on TGF β -induced fibroblast activation. Consistent with an antifibrotic role of 12/15-LO and 12/15-LO-derived eicosanoids, the tested mediators, which included 12-HETE, 13-HODE, 15-HETE and LXA₄, interfered with TGF β -induced activation of the different MAP-kinase pathways (figure 5A,B). Likewise, the increased TGF β -induced expression of collagen 1a2 mRNA in 12/15-LO-deficient fibroblasts was significantly reduced by these eicosanoids (figure 5C). Together these data identify both 12/15-LO and its metabolites as important regulators of fibroblast activation.

DISCUSSION

Although several molecular pathways that stimulate fibrosis have been identified over recent years, little is known about endogenous protective feedback loops limiting fibroblast activation and fibrosis. Our study provides evidence for a role of the 12/15-LO pathway as endogenous and negative regulator in experimental fibrosis. While we observed an increased formation of the 12/15-LO product LXA₄ in the skin of bleomycin-treated animals, absence of 12/15-LO drastically reduced formation of this eicosanoid. This was paralleled by a drastic increase in the progression of dermal fibrosis. As expected, LXA₄ was not completely absent as enzymes other than 12/15-LO can account for LXA₄ production.⁵ These bone marrow transfer studies demonstrated that the antifibrotic effect of 12/15-LO is due to expression of this enzyme in mesenchymal rather than haematopoietic cells. This highlights that fibroblasts and/or other mesenchymal cells are key players in the antifibrotic 12/15-LO pathway. In line with these results, we observed an exacerbation of fibrosis also in the *tsk-1* model of dermal fibrosis, which is a model of less inflammation-dependent stages of skin fibrosis that are mainly driven by endogenous activation of fibroblasts.

Figure 5 12/15-LO-derived eicosanoids counteract TGFβ induced activation of fibroblasts. (A) Primary dermal fibroblasts of 12/15-lipoxygenase (12/15-LO) mice were pretreated for 4 h with the indicated eicosanoid and stimulated with transforming growth factor β (TGFβ; 10 ng/ml) for 5 h before phosphorylation of the indicated mitogen-activated protein (MAP)-kinase pathways was analysed by Western Blot. Intensities of the respective p-MAPK and their corresponding β-actin bands were measured and are depicted as mean p-MAPK/β-actin ratios (B). (C) After 5 h of pretreatment with the indicated eicosanoids, fibroblasts were stimulated with TGFβ (10 ng/ml) for 8 h before mRNA expression of col 1a2 was determined by real-time PCR.

Our in vitro data further confirm the important role of 12/15-LO in the response of fibroblasts to TGFβ. 12/15-LO-deficient fibroblasts showed an enhanced expression of col 1a2. Moreover, 12/15-LO-deficient fibroblasts showed an aberrant signalling response to TGFβ, which was characterised by an increased activation of different MAP-kinase pathways including JNK, p38 and ERK. These pathways have been previously implicated in the activation and proliferation of fibroblasts.^{24–26} In turn, 12/15-LO products, including 12-HETE, 13-HODE, 15-HETE and LXA₄, were able to differentially block these intracellular signalling pathways after stimulation with TGFβ and reduce the expression of col 1a2. Together these data provide evidence for a feedback loop involving 12/15-LO and 12/15-LO-derived eicosanoids, which limits the fibrotic tissue response. The finding that a deficiency of 12/15-LO exacerbates fibrosis in bleomycin-induced dermal fibrosis and in the tsK-1 model suggests that this feedback loop may be relevant both during early, inflammatory phases and later, in the non-inflammatory stages of fibrotic diseases.

Our results are consistent with a previous report that lipoxin analogues prevent experimental pulmonary fibrosis.²⁷ Again, these protective effects seem to be linked both to anti-inflammatory and direct antifibrotic effects of these eicosanoids, because lipoxins have been shown to block the activation of pulmonary fibroblasts in vitro.²² Interestingly, other eicosanoids such as leukotrienes clearly have opposing and profibrotic effects.⁷ This indicates a complex crosstalk, which controls the fibrotic tissue response on the level of arachidonic acid metabolism.

The clinical relevance of lipoxins and leukotrienes for SSc-associated fibrosis is highlighted by studies on the lipid profile in the bronchoalveolar lavage of patients with SSc. Significantly decreased levels of LXA₄ were measured in SSc, whereas the

concentration of leukotrienes was increased.^{8,9} Together, these results suggest that a shift in the balance of these profibrotic and antifibrotic lipid mediators may contribute to the pathogenesis of SSc by stimulating fibroblast activation and progression of fibrosis.

Together our data identify the 12/15-LO-LXA₄ pathway as a negative key regulator of the fibrotic tissue response, rendering it a potential target for therapeutic intervention in other fibrotic diseases. Given that lipoxins are currently being explored as new therapeutic agents for inflammatory diseases, these findings may have direct translational implications. However, further studies with exogenous lipoxins in preclinical models of fibrosis are warranted.

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