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ACTIVATION OF SOMATOSTATIN RECEPTORS ATTENUATES PULMONARY FIBROSIS

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ABSTRACT

Somatostatin analogs may have antifibrotic properties in the lung. The aim of this study was to evaluate the expression of the five somatostatin receptors sst1 to sst5 in the normal and fibrotic mouse lung and the action of SOM230 (pasireotide), a new somatostatin analog with a long half life, in bleomycin-induced lung fibrosis and in human lung fibroblasts *in vitro*. After intratracheal injection of bleomycin, C57Bl6 male mice received one daily sub-cutaneous injection of SOM230 or saline. The lungs were evaluated on day 3, 7 and 14 after bleomycin. We found that all the somatostatin receptors were expressed in the normal mouse lung. Sst2 receptor mRNA expression was increased after bleomycin. SOM230 improved mice survival (69% vs 44%, P=0.024) and reduced lung collagen content at day 14, and decreased lung collagen-1 mRNA at day 7. SOM230 reduced the BAL inflammatory cells influx at day 3, decreased lung CTGF mRNA and TGF-β mRNA, and increased lung hepatocyte growth factor and keratinocyte growth factor mRNA. The sst2 receptor was strongly expressed in the human lung, either normal or fibrotic, particularly by fibroblasts. *In vitro*, SOM230 reduced BrdU incorporation by control human lung fibroblasts cultured in basal conditions or with TGF-β, and reduced alpha-1 collagen-1 mRNA expression in TGF-β stimulated fibroblasts. We conclude that SOM230 attenuates bleomycin-induced pulmonary fibrosis in mice and human lung fibroblasts activation. This study points to a potential new approach for treating pulmonary fibrotic disorders.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease which carries a poor prognosis. To date, no treatment has been demonstrated to prevent the evolution of fibrosis in IPF patients and new therapeutic opportunities are needed. While the pathophysiology of pulmonary fibrosis is poorly elucidated, it is characterised at the microscopic level by fibroblasts accumulation and extracellular matrix deposition in the lung, leading to alveolar wall thickening and loss of elasticity. Apart from chronic lung inflammation, which probably participates in the pathophysiology of the disease [1], the recruitment, activation and proliferation of fibroblasts are now considered key features during the development of pulmonary fibrosis and therapeutic agents targeting fibroblasts are being evaluated in idiopathic pulmonary fibrosis [2].

Somatostatin is an endogenous cyclic peptide initially identified as a regulator of Growth Hormone secretion. Five somatostatin receptors, named sst1, sst2A, sst3, sst4 and sst5, have been described in humans, that equally bind somatostatin. Although the relative expression and the localisation of these receptors vary within organs, their expression in the lung has been poorly studied. In a previous study, our group has shown that human lung fibroblasts, from normal and fibrotic lung, bound radiolabeled somatostatin *in vitro* [3]. We also evidenced an increased uptake of radiolabeled octreotide (a somatostatin agonist) in the lung in patients with IPF when compared to control patients [3]. Most importantly, the octreotide uptake correlated with the severity of lung fibrosis [3]. Altogether, these data suggested that somatostatin receptors were increased in IPF lungs.

Somatostatin and its pharmacological analogs display anti-fibrotic activities *in vitro* and *in vivo* in different experimental settings [4]. The pathways involved in this antifibrotic effect are not completely elucidated but inhibition of transforming growth factor-β1 (TGF-β1) production [5, 6] as well as a degree of anti-inflammatory actions [7] may contribute to the

protective effect. However, the antifibrotic effect of somatostatin and its analogs has never been evaluated in the lung.

SOM230 (pasireotide) is a new somatostatin analog that binds four out of the five sst receptors (sst1, sst2A, sst3 and sst5) and has a long half-life which makes it suitable for a therapeutic use [8]. In this study we evaluated the antifibrotic effect of SOM230 in a bleomycin-induced lung fibrosis model in mice. We quantified the expression and localization of somatostatin receptors (sst1 to sst5) in the normal and fibrotic lung, and we evaluated whether SOM230 modulated the TGF-β and CTGF profibrogenic pathways. Furthermore, we characterized the effect of SOM230 on human lung fibroblasts *in vitro*.

MATERIAL AND METHODS

Bleomycin animal model

Male C57BL/6J mice (R. Janvier, Le Genest St Isle, France), aged 6-7 weeks, were kept in accordance with INSERM rules. On day 0, the mice were administered 80 µg bleomycin hydrochloride (Bleomycine Bellon, Aventis, Paris, France) intratracheally, and were randomly assigned to receive either SOM230, 25 µg/kg/day (bleo+SOM mice) or 2.5 µg/kg/day, or an equal volume of saline (bleo+vehicle mice). SOM230 was diluted in 100 µl saline and sub-cutaneously administered daily beginning immediately after bleomycin injection. Mortality was assessed daily over a 14 days-period. For further analysis, animals were sacrificed on day 3, 7 or 14 after bleomycin admnistration.

To evaluate the effect of late treatment, SOM230 (25 µg/kg/day) or vehicle was given from day 5 to day 13. The animals were sacrified at day 14. Naïve mice were used as controls. The doses of SOM230 used in this study were similar to those used in previous experiments [9].

In further experiments, controls animals received SOM230 (25µg/kg/day) or vehicle for 3, 7 or 14 days, and were sacrificed at each time point (N=6 per time).

Tissue sampling, lung histology, TGF-β1 assay and mRNA analysis

At specified time points, the animals were euthanised and the lungs were freed of blood by perfusion. Bronchoalveolar lavage (BAL) was performed with 1ml of saline. Total and differential cell counts were estimated. BAL supernatant was stored at –80°C until TGF-β1 assay.

Total TGF-β1 was measured after acidification of the samples according to the manufacturer instructions (Quantikine®, R&D System Europe, Lille, France).

Semi-quantitative assessment of lung injury used the grading system described by Inoshima and colleagues [10].

Lung collagen was quantified in snap-frozen right lungs with the Sircol collagen assay (Biocolor Ltd, Newtownabbey, UK).

RNA extraction and real time quantitative PCR were performed on left mouse lungs using standard protocols (see on line supplement). The expression of the gene of interest was expressed as a ratio to the Ribosomal Protein L13 (RPL13) gene as previously described [11].

Immunohistochemical detection of somatostatin receptors

The sst2 receptor was detected by immunohistochemistry on mice samples with a rabbit polyclonal anti-mice sst2A antibody (ref SS-800, Biotrend, Cologne, Germany) (1/400 dilution). Immunodetection was performed on frozen human lung tissue samples (from 3 controls and 5 patients with IPF, diagnosed according to the ATS/ERS consensus criteria [12]-the origin of lung samples has been previously described [11]**)** and cultured human

fibroblasts, using a polyclonal rabbit antibody to human somatostatin receptor type 2B (ref SS-860, Biotrend) (1/1000 dilution). Standard protocols were used (see on line supplement).

Data analysis.

Data were expressed as median [extremes values]. Data analysis was performed with the GraphPad Prism 4.0 software (GraphPad software, San Diego, USA). Survival between experimental and control groups was studied with a Kaplan-Meier analysis using the log rank test. Experimental groups were compared first with a non parametric analysis of variance equivalent (the Kruskall-Wallis test) and if significant, pairwise comparisons were performed with the Mann-Whitney U test. Comparison of histological scores on day 14 was performed with the Fisher exact test after grouping the scores 0 and 1 and the scores 2 and 3. *P* values below 0.05 were considered significant.

RESULTS

Somatostatin receptors are present in the lung and their expression is modulated after bleomycin instillation

In the naive mice, all somatostatin receptors (sst1 to sst5) mRNA were detected in the lung, with very low levels of expression of sst5 (figure 1). After bleomycin instillation, the expression of sst2A and sst5 mRNA was strongly increased, with a maximum at day 7, whereas the expression of sst1 and sst4 mRNA was decreased (figure 1).

Treatment with SOM230 of bleomycin-injected mice temporarily increased the expression of sst1 (sst1/RPL13 mRNA ratio= 2.9 [2.5-24] on day 7 in bleo+SOM mice vs 0.9 [0.4-3.5] for bleo+vehicle mice, n=4, *P*=0.015), and partly inhibited the increase of expression of sst2A (sst2/RPL13 mRNA ratio= 7.4 [2.8-10.6] on day 7 in bleo+SOM mice vs 19 [14.1-36.3] for bleo+vehicle mice, n=4, *P*=0.015) (figure 1).

We characterized the cellular localization of the sst2A receptor by immunohistochemistry. In the normal murine lung, sst2A was localized to alveolar type 2 pneumocytes, bronchial epithelial cells, alveolar macrophages, arterial smooth mucle cells and some endothelial cells. After bleomycin instillation, the number of sst2A-positive cells increased, with a maximum at day 7, essentially because of an increased infiltration of sst2A-positive cells (macrophages and monocytes) in the inflammatory areas (figure 2).

Treatment with SOM230 improved mice survival after bleomycin instillation.

Intra-tracheal injection of bleomycin induced a significant mortality among mice, which typically occurred from day 7. Treatment with SOM230 (25 µg/kg/day) increased the 14 days survival of mice instilled with bleomycin to 69% (n=36) versus 44% in the bleo+vehicle group (n=41, *P*=0.02). Treatment with SOM230 at a lower dose (2.5 µg/kg/day) did not improve survival (14 days survival: 32%, n=22, *P*=0.46) (figure 3).

Treatment with SOM230 attenuated bleomycin-induced pulmonary fibrosis.

We analyzed the histological changes in the lung of surviving mice at day 14 following bleomycin instillation in bleo+SOM mice (25 µg/kg/day, n=11; 2.5 µg/kg/day, n=7) compared to bleo+vehicle mice. As shown in figure 4, the treatment with SOM230 25 µg/kg/day improved the pathological score at day 14, when compared to bleo+vehicle mice (*P*=0.03) whereas the lower dose (2.5 µg/kg/day) had no effect. In view of these favorable results, the 25 µg/kg/day dose of SOM230 was used in further experiments.

SOM230 reduced lung collagen concentration

Treatment with SOM230 reduced lung collagen content on day 14 (112 µg/lung [21-146] in bleo+SOM mice versus 139 µg/lung [90-153] in the bleo+vehicle mice, *P*=0.01) compared to

55 µg/lung [45-90] in the naive mice (*P*=0.007 and *P*=0.003 versus bleo+SOM mice and bleo+vehicle mice respectively) (figure 4).

In the bleo+vehicle mice, alpha-2 collagen-1 (COL1A2) mRNA lung content was increased at day 3 after bleomycin instillation, peaked at day 7, and then decreased by day 14 (figure 4). SOM230 treatment strongly inhibited the peak of COL1A2 mRNA expression in the lung at day 7 (COL1A2/RPL13 mRNA ratio = 2.6 in the bleo+SOM mice versus 23 in the bleo+vehicle mice, *P*=0.01). SOM230 had no effect on COL1A2 mRNA contents at other time points. These results show that the reduction of collagen content at day 14 was preceeded by a reduction in COL1A2 mRNA at day 7.

Treatment with SOM 230 reduced lung inflammation and inhibited the increase of TGF-β1 and CTGF

Treatment with SOM230 reduced the alveolar inflammatory cells influx at early times as assessed by the reduction of total BAL cellularity, BAL macrophages and BAL lymphocytes counts on day 3 (Table 1). On day 7 and day 14, BAL cellularity was similar in bleo+SOM and bleo+vehicle mice (data not shown).

Table 1: Analysis of BAL cellularity in control naive mice and at day 3 after intratracheal bleomycin injection in mice treated with saline or SOM230.

	N	Cells /µl	Macrophages /µl	Lymphocytes /µl	Neutrophils/µl
Controls	4	230 [100-240]	224 [88-237]	5 [0-12]	0 [0-0]
Day 3					
Saline	6	602 [490-1050]	237 [192-300]	292 [151-409]	115 [66-280]
SOM230	6	365 [40-540] *	130 [16-300]	68 [12-221] *	141 [12-161]

Data are medians[extremes]; *$P<0.05$*

TGF-β and connective tissue growth factor (CTGF) are two potent pro-fibrogenic factors. Lung TGF-β mRNA content tended to be lower in bleo+SOM mice on day 7 after bleomycin administration when compared with bleo+vehicle mice (TGF-β/RPL13 mRNA ratio : 2,6 [0,9-3,2] in bleo+SOM mice versus 4,8 [2,3-16,1] in bleo+vehicle mice, P=0.11). TGF-β1 concentration in BAL fluid was lower in bleo+SOM mice at day 14 (85 pg/ml [64-121]) when compared to bleo+vehicle mice (103 pg/ml [96-206], n=9, P=0.01) (Figure 5).

Similarly, SOM230 strongly inhibited the increase of CTGF mRNA induced by bleomycin. After bleomycin instillation, CTGF mRNA contents were increased by day 3, with a maximum at day 7. Treatment with SOM230 reduced by 70% the CTGF/RPL13 mRNA ratio at day 7 (ratio = 6.4 [4.7-8.3], n=5) when compared with bleo+vehicle mice (ratio=22.2 [14.6-29.2], n=4, P=0.01) (figure 5).

Furthermore, SOM230 promoted the expression of hepatocyte growth factor (HGF) and keratinocyte growth factor (KGF) (two antifibrotic mediators) after bleomycin, and reduced lung CTGF mRNA content by 30% when given to naive mice (see on line data supplement).

Effect of late treatment with SOM230

In a further set of experiments, we assessed the protective effect of SOM230 given daily later after bleomycin instillation. Mice were started with SOM230 (25 µg/kg/day) from day 5 after bleomycin instillation until day 13.

In these experiments, the pathological score (score > 1: 5/9 bleo+vehicle animals versus 3/8 bleo+SOM animals) and lung collagen content (79.4 µg/lung [61-128] in bleo+SOM mice versus 93.4 µg/lung [56-130] in bleo+vehicle mice) tended to be lower in bleo+SOM mice without reaching statistical significance. TGF-β mRNA and TGF-β BAL concentration were

strongly reduced in the bleo+SOM mice on day 14 (figure 6). There was no significant effect on lung CTGF mRNA content on day 14.

Immunohistochemical detection of the sst2 receptor in human lung (Figure 7).

Expression of the sst2 receptor was very low in the normal lung, and was essentially detected in alveolar macrophages and in isolated alveolar cells primarily located in the corners of the alveoli, representing most probably type 2 pneumocytes. The receptor was not detected in endothelial cells. In the fibrotic lung, we observed a strong expression of this receptor in hyperplastic alveolar epithelial cells, endothelial cells and fibroblasts as well as a predominance of the expression of the sst2 receptor in areas of dense fibrosis.

In vitro, fibroblasts cultured from normal lung and fibrotic lung strongly expressed the sst2 receptor as assessed by immunohistochemistry (Figure 7). The intensity of labeling was similar in the two groups.

DISCUSSION

To our knowledge, this is the first study describing the anti-fibrotic action of a somatostatin analog in pulmonary fibrosis. Our results demonstrate *1)* that somatostatin receptors are expressed in the lung and are increased during bleomycin-induced lung fibrosis in mice and in IPF in humans, and *2)* that SOM230, a new pharmacological analog of somatostatin, has a protective effect in bleomycin-induced pulmonary fibrosis in mice, as daily treatment with SOM230 improved survival, reduced the lung pathological score and lung inflammation, reduced lung collagen contents and lung collagen mRNA expression, inhibited the expression of profibrotic mediators (TGF-β and CTGF) and increased the expression of two antifibrotic mediators (HGF and KGF). Although less marked, the antifibrotic effect of SOM230 was also apparent when treatment with SOM230 began 5 days

after bleomycin instillation. This suggests that SOM230 may have a therapeutic effect on an ongoing fibrotic process. Furthermore, in *in vitro* studies (see on line supplement), the incubation with SOM230 inhibited the proliferation and collagen-1 mRNA expression of human lung fibroblasts.

Emerging data suggest that somatostatin may have interesting antifibrotic properties, both *in vitro* and *in vivo*, which we have confirmed in this study using an *in vivo* model of lung fibrosis.

First, we demonstrated the expression of somatostatin receptors in the lung. All sst receptors mRNA were detected in mice lung, although the sst5 receptor was expressed at a very low level. Interestingly, sst receptors mRNA expression appeared to be differentially modulated following bleomycin-induced lung fibrosis: the expression of sst2A and sst5 mRNA was strongly increased, the expression of sst1 and sst4 mRNA was decreased, whereas the expression of sst3 mRNA remained unchanged. The increased expression of the sst2 receptor is of paramount importance since the sst2 receptor is thought to play a prominent role in the biological effects of somatostatin analogs [13]. Increased mRNA expression was associated with an increase of the sst2 positive cells on immunohistochemistry. The later results are reminiscent of our previous report of increased uptake of radiolabeled octreotide in patients with IPF [3].

Interestingly, both inflammatory cells and resident lung cells expressed the sst2 receptor in mice and human samples as detected by immunohistochemistry. In the bleomycin mouse model, the increased expression of sst2 was due, at least in part, to an influx of inflammatory cells, particularly monocytes and macrophages. The expression of sst2 has been shown to be upregulated in activated monocytes [14]. Our results support data indicating that somatostatin analogs exert anti-inflammatory effects on lymphocytes, macrophages and

neutrophils [15-20]. Interestingly, SOM230 reduced the alveolar inflammatory cells influx on day 3, demonstrating an anti-inflammatory action of the compound in this model.

Although somatostatin and its analogs have shown interesting antifibrotic properties in a number of animal models, they have never been tested previously in a model of pulmonary fibrosis. Octreotide is the most extensively studied molecule in this field. In rats, octreotide inhibits the development of liver fibrosis induced by extrahepatic biliary obstruction or carbon tetrachlorure poisoning [21, 22], inhibits esophageal or digestive fibrosis induced by radiation [6, 23], and decreases the accumulation of connective tissue in a murine model of hepatic schistosomiasis [24]. Somatostatin and its analogs develop their antifibrotic action through different mechanisms. Somatostatin has been shown to promote fibroblasts apoptosis *in vitro* through its sst2 receptor [25] whereas octreotide inhibits the proliferation of skin and orbital fibroblasts [26, 27]. *In vitro*, somatostatin modulates fibroblasts proliferation through the activation of the ERK and MAPkinase pathways [4]. We show in this study that SOM230 inhibits human lung fibroblasts proliferation *in vitro*. Some data suggest that somatostatin analogs may act through an inhibition of TGF-β production [5, 23, 28] although TGF-β inhibition was not apparent in all models studied [6]. TGF-β is the prototypical profibrotic mediator in the lung. TGF-β has been shown to increase the expression of the sst2 receptor in mice, through a Smad4-dependent pathway [29]. Broad expression of sst receptors in the lung indicates that beside the fibroblasts, SOM230 may have many cellular targets, both on resident cells and inflammatory cells, all possibly contributing to its protective action.

Interestingly, SOM230 seems to downregulate macrophages and lymphocytes lung infiltration on day 3, but not neutrophils (table 1). This phenomenon could be due to a direct effect of SOM230 on leukocytes survival. Indeed, somatostatin inhibits proliferation and promotes apoptosis in lymphocytes [30] and reduces macrophages viability [31], but such an effect has not been reported with neutrophils. SOM230 could also differentially modulate the

expression of chemoattractants and adhesion molecules. We did not specifically adress this point. In one study, somatostatin did not modify neutrophil migration in vitro in response to different stimuli [32].

The protective effect of SOM230 in our study is consistently associated with a decrease of TGF-β1 expression, observed both when SOM230 was given from day 0 to day 14, or when given later, from day 5 to day 14. Reduced expression of TGF-ß1 may contribute to the strong inhibition of lung CTGF expression that we observed on day 7 since CTGF expression is selectively induced by TGF-β1 [33]. In vitro, octreotide, a somatostatin analog, inhibits the expression of TGF-β and CTGF mRNA by hepatic stellate cells [34]. Whether somatostatin analogs modulate directly CTGF expression is unknown but may be supported by our observation that SOM230 reduced lung CTGF mRNA in control mice treated with SOM230.

Interestingly, our results suggest that SOM230 could exert its antifibrotic properties through the increased expression of antifibrotic mediators, such as HGF and KGF (see on line supplement). HGF and KGF are produced mainly by fibroblasts [35]. Our group has previously shown that human lung fibroblasts cultured from the IPF lung have a reduced capacity to secrete HGF and KGF [36, 37] and to activate proHGF [11]. Reduced expression of TGF-β1 by SOM230 explain the increase in HGF and KGF expression since TGF-β1 is a known inhibitor of HGF and KGF expression [35].

We observed that endothelial cells expressed the sst2 receptor in the lung, both in mice and in human IPF lung. This result is in agreement with previously reported evidence of the effect of SOM230 in reducing endothelial cell proliferation *in vitro* [38] and its action as an anti-angiogenic drug [39]. This is particularly interesting since increased vascularity is a hallmark of IPF [40] and reduction of aberrant angiogenesis reduces the development of

bleomycin-induced pulmonary fibrosis [41]. These findings could further explain the antifibrotic effect of SOM230.

We also have shown that SOM230 inhibits collagen-1 mRNA expression by human lung fibroblasts stimulated *in vitro* with TGF-β1 (see on line data supplement). Interestingly, the maximal inhibition was obtained with low concentrations of SOM230 (10^{-10}M), that are likely to be obtained *in vivo*. Somatostatin has been previously shown to directly inhibit the expression of collagen and alpha-smooth muscle actin in hepatic stellate cells [42]. In that study, the inhibition of collagen expression was also obtained only with low concentrations of somatostatin (10^{-9}M); the authors attributed this effect to desensitization of somatostatin receptors with higher doses [42].

Altogether, the encouraging results of this study indicate that SOM230 exert a protective antifibrotic action *in vivo* in the bleomycin model. SOM230 demonstrates its antifibrotic action, both through and anti-inflammatory effect and a direct effect on lung fibroblasts as evidenced our *in vitro* results obtained on human lung fibroblasts. The encouraging results obtained from this study provide a rationale for clinical trials based on somatostatin analogs in patients with pulmonary fibrosis.

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FIGURE LEGENDS

Figure 1 : Lung expression of somatostatin receptors .

Panels A to E **:** Somatostatin (sst) receptors mRNA were quantified by quantitative PCR in the lung of naive mice (controls), or in mice who received intra-tracheal bleomycin and were sacrificed on day 3, day 7 or day 14 after bleomycin, and were treated daily with a subcutaneous injection of SOM230 (25 µg/kg/day) (SOM) or vehicle. mRNA content was expressed as the ratio to the housekeeping gene RPL13 (n = 4 to 7 animals for each experimental condition). All somatostatin sst receptors mRNA were present in the lung of control mice, but with a very low level of expression of sst5 (panel E). After bleomycin instillation, there was an increased expression of sst2A (panel B) and sst5 in bleo+vehicle mice, with a maximum on day 7, and a decreased expression of sst1 (panel A) and sst4 (panel D). The expression of sst3 mRNA remained unchanged (panel C). After bleomycin instillation, SOM230 treatment temporarily increased the expression of sst1, partly inhibited the increased expression of sst2A, and did not modify the expression of sst3, sst4 and sst5. * *P<0.05.*

The centerline of the box denotes the median, the extremes of the box denote the interquartile range, and the bars denote the highest and lowest values.

Figure 2 : Immunohistochemical detection of somatostatin receptor sst2A in naive mouse lung and after bleomycin instillation.

In normal lung (a) sst2A was expressed by alveolar macrophages, epithelial bronchial cells, arterial and bronchial smooth muscle cells, some endothelial cells and some type 2 pneumocytes (inset, arrow) After bleomycin instillation, the number of sst2A-positive cells increased on day 3 (b) with a maximum on day 7 (c, d) and then decreased on day 14 (e). The

sst2A positive cells were essentially localised in the inflammatory areas. (Original magnification: a, b, c, e: x80; d: x 160; inset : x400).

Figure 3 : SOM230 improves mice survival after bleomycin instillation.

Treatment with SOM230 (25µ/kg/day, dashed line) improved survival compared to the bleo+vehicle group (continuous line) (69% vs 44%, *P<0.05*). Treatment with SOM230 at a lower dose (2.5 µg/Kg/day, dotted line) did not improve survival (32%) (n=22 to 41 animals for each experimental condition). **P< 0.05*.

Figure 4 : SOM230 reduces lung fibrosis after bleomycin instillation.

Representative lung sections in a bleo+vehicle mice (panel A) and in a bleo+SOM mice (25 µg/kg/day) (panel B) on day 14 after bleomycin intratracheal injection (hematoxylin-eosin-staining, original magnification: x10). Histological lesions were scored from 0 to 3 (see methods). The percentage of lungs with a lung fibrosis score higher than 1 (lesions involving more than 25% of the lung) is shown in panel C (n = 7 to 11 animals per group). SOM230 (25 µg/kg/day) improved the pathological score at day 14 whereas SOM230 at a lower dose (2.5 µg/kg/day) had a small not statistically significant effect.

Lung collagen content was measured by the Sircol assay and expressed as µg collagen/right lung (panel D). Lung collagen content was increased 14 days after bleomycin instillation in bleo+vehicle mice compared to naive control mice. SOM230 reduced total lung collagen content compared to vehicle (n=5 to 12 for each experimental condition).

Alpha-2 collagen-1 (COL1A2) mRNA expression was assessed by quantitative real time PCR and expressed as the ratio to RPL13 mRNA content in the lung of naive mice (controls) and at day 3, day 7 and day 14 after bleomycin instillation (panel E). In bleo+vehicle mice COL1A2

mRNA lung content was increased at day 3, peaked at day 7 and decreased at day 14. SOM230 treatment inhibited the peak of COL1A2 mRNA expression at day 7. SOM230 had no effect at other time points (n=5 to 7 animals for each experimental condition). *$P<0.05$.

The centerline of the box denotes the median, the extremes of the box denote the interquartile range and the bars denote the highest and lowest values.

Figure 5 : SOM230 promotes an anti-fibrotic environment in the lung.

Panel A: At day 14, SOM230 reduced TGF-β1 concentration in BAL fluid compared to bleo+vehicle animals and control naive mice.

Panel B: CTGF mRNA content was assessed by quantitative real time PCR and expressed as the ratio to RPL13 mRNA content in the lung of naive mice (controls) and at day 3, day 7 and day 14 after bleomycin instillation. In bleo+vehicle mice CTGF mRNA content was increased at day 3 with a maximum at day 7. SOM230 treatment inhibited the peak of CTGF mRNA expression at day 7. SOM230 had no effect on CTGF mRNA content at other time points.

The centerline of the box denotes the median, the extremes of the box denote the interquartile range and the bars denote the highest and lowest values. (n=5 to 9 animals for each experimental condition). * $P<0.05$

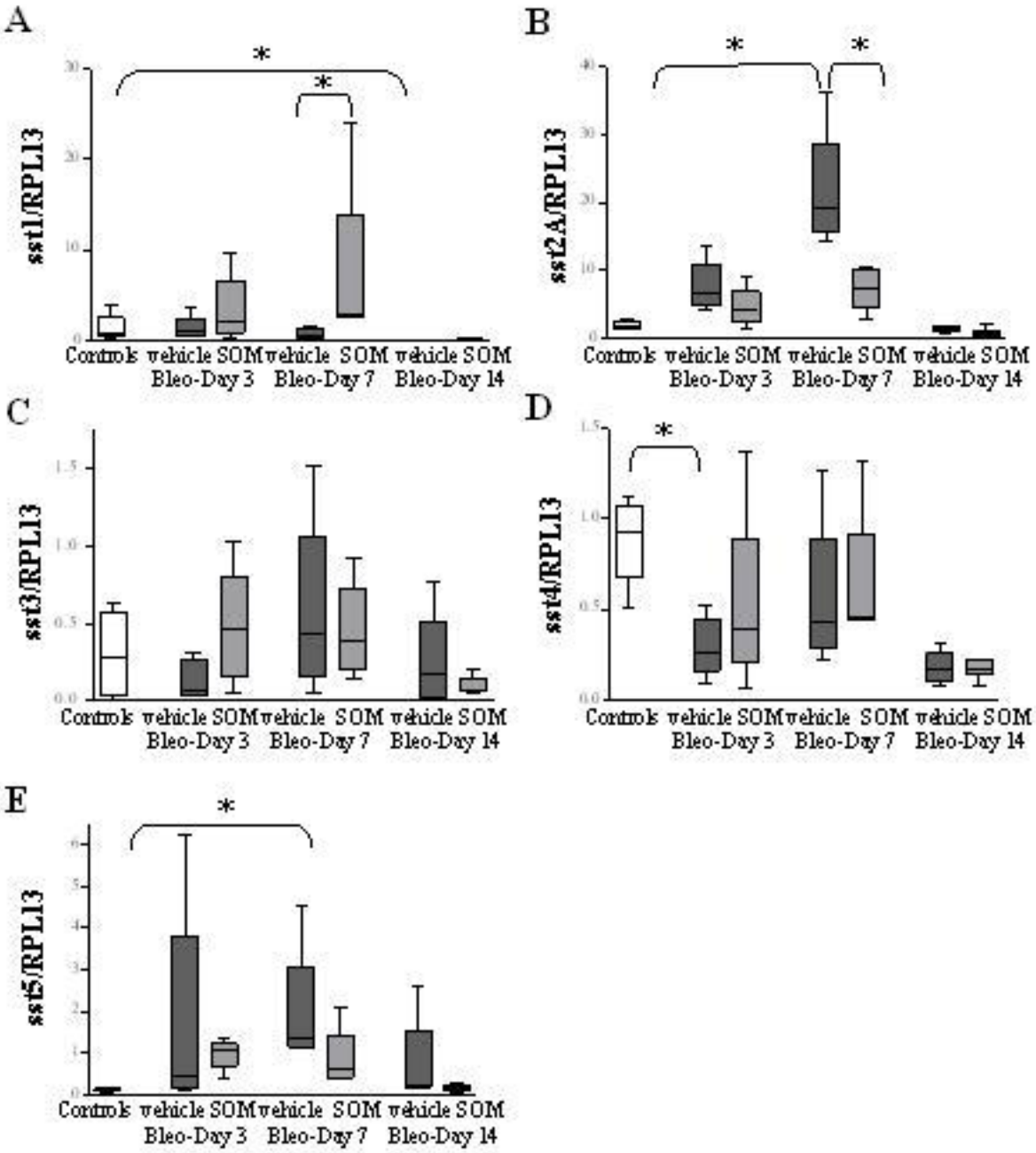
Figure 6 : TGF-β1 concentration in BAL fluid in late treatment with SOM230.

SOM230 (25 µg/kg/day), or vehicle was given from day 5 to 13. At day 14, SOM230 reduced TGF-β1 mRNA (panel A) and TGF-β1 concentration in BAL fluid (panel B) compared to vehicle (n=5 to 9 animals for each experimental condition). *$P<0.05$

Figure 7 : Immunohistochemical detection of the type 2 somatostatin receptor (SST2) in human lung.

Expression of SST2 receptor was very low in the normal lung (a), and was essentially detected in alveolar macrophages (AM) and in isolated alveolar cells primarily located in the corners of the alveoli, probably type 2 pneumocytes (arrows). In usual interstitial pneumonia samples (b), we observed a strong expression of the receptor in hyperplastic alveolar epithelial cells, endothelial cells (v), fibroblasts, lymphoid aggregates (Ly) and alveolar macrophages. The inset shows a high power view of positive fibroblasts (arrow). Lung fibroblasts cultured from normal lung (c) and from usual interstitial pneumonia (d) expressed the SST2 receptor. (Original magnification: a, b x 250; inset, C and D x 400).

Figure 1



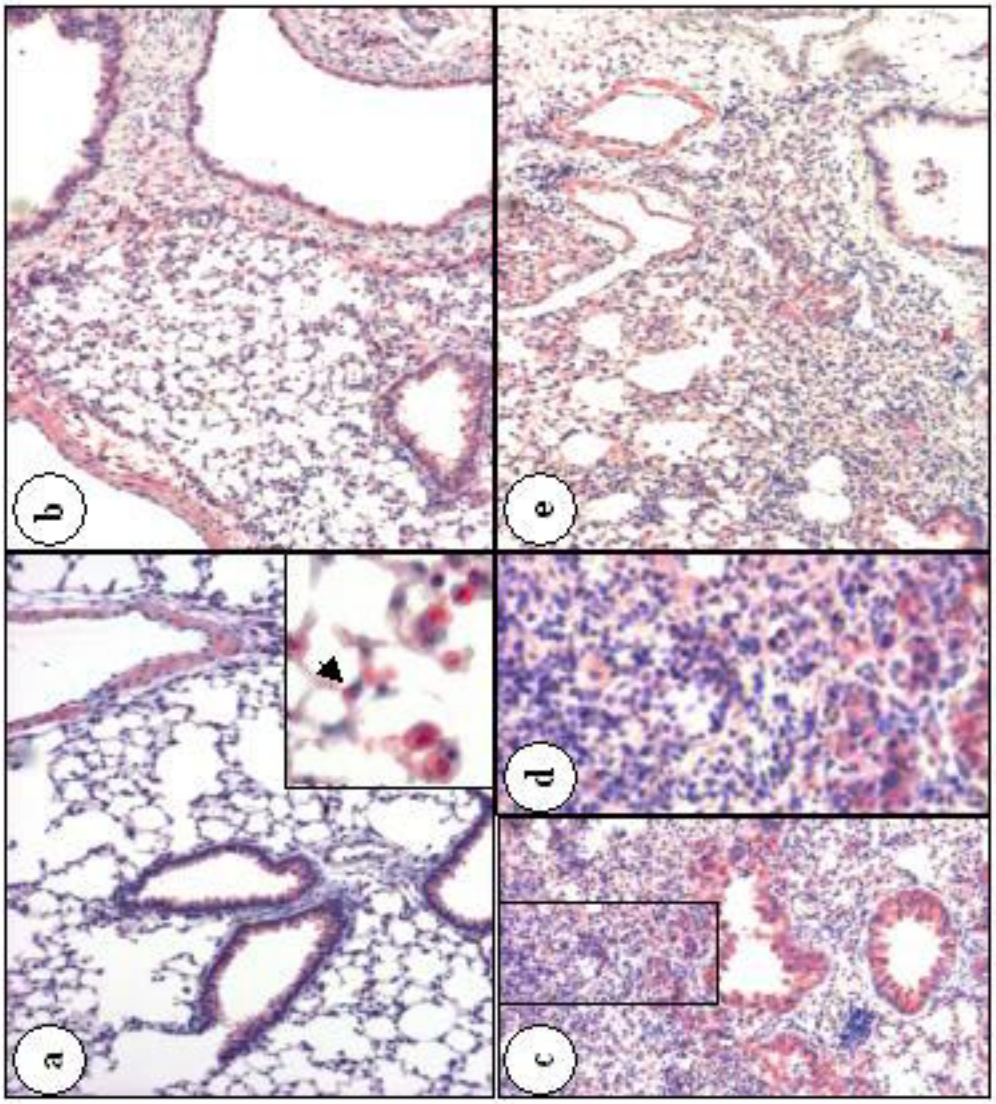


Figure 2

Figure 3

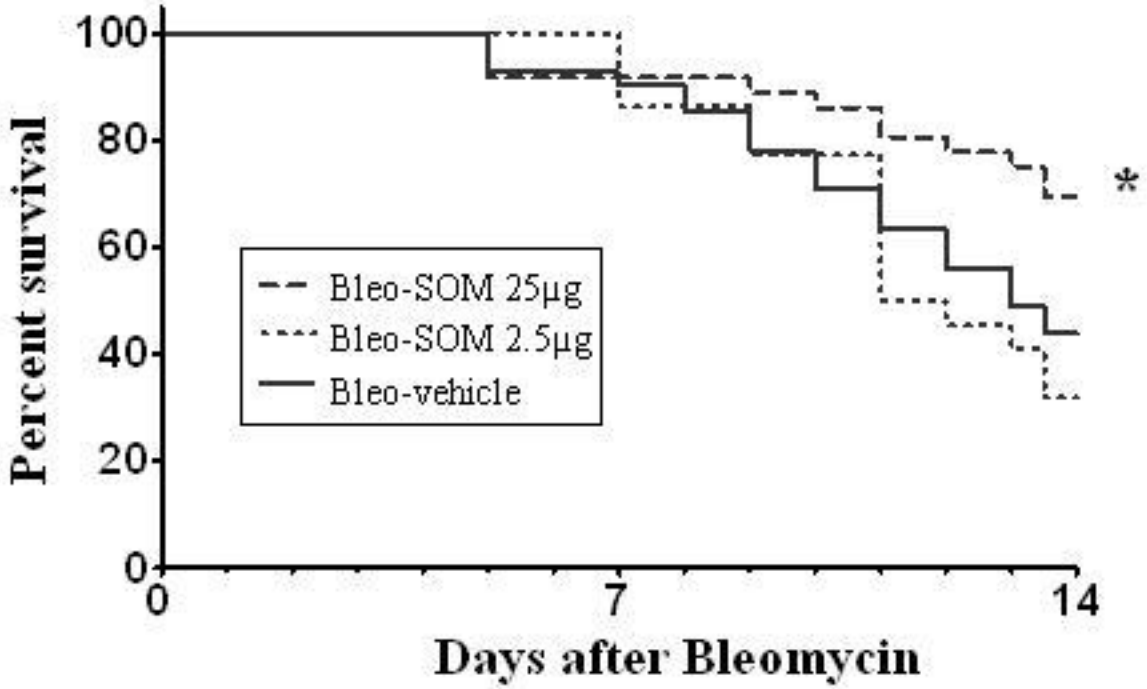
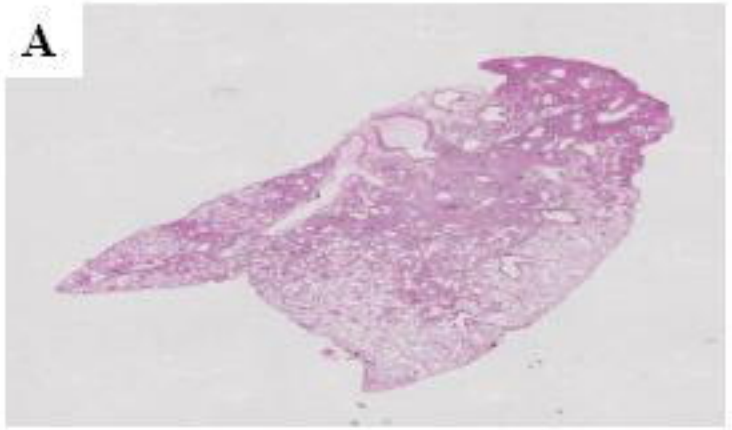
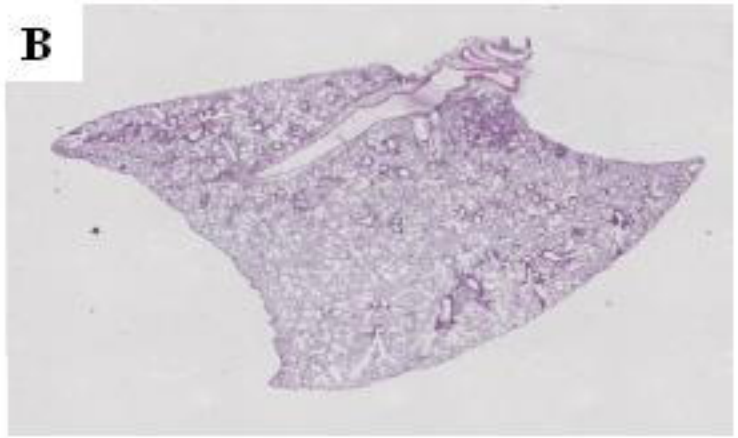
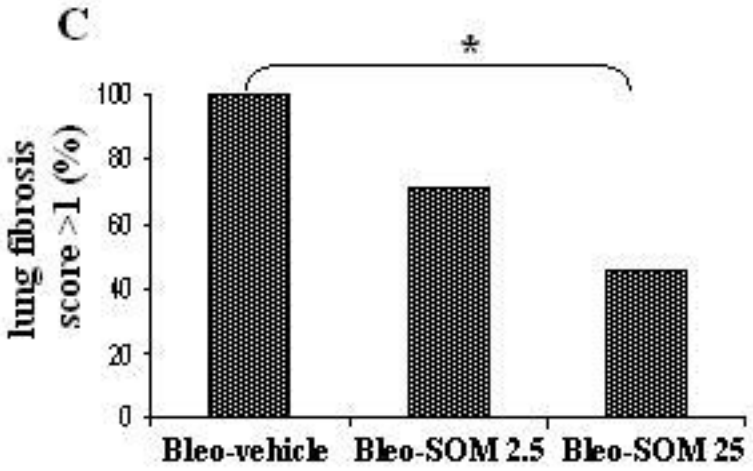
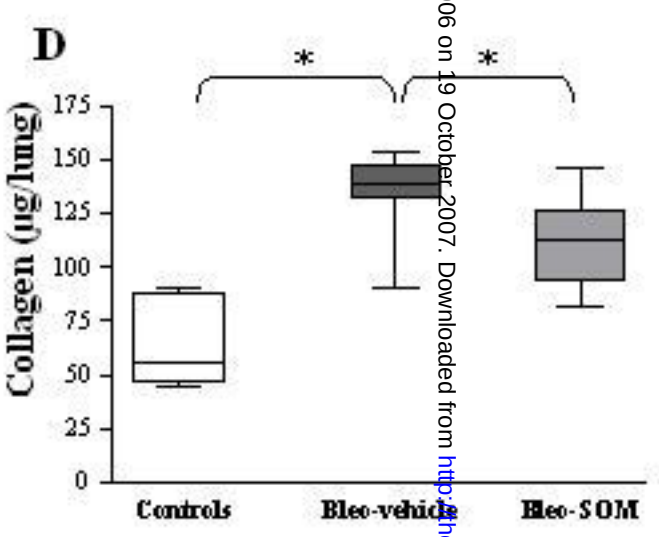


Figure 4









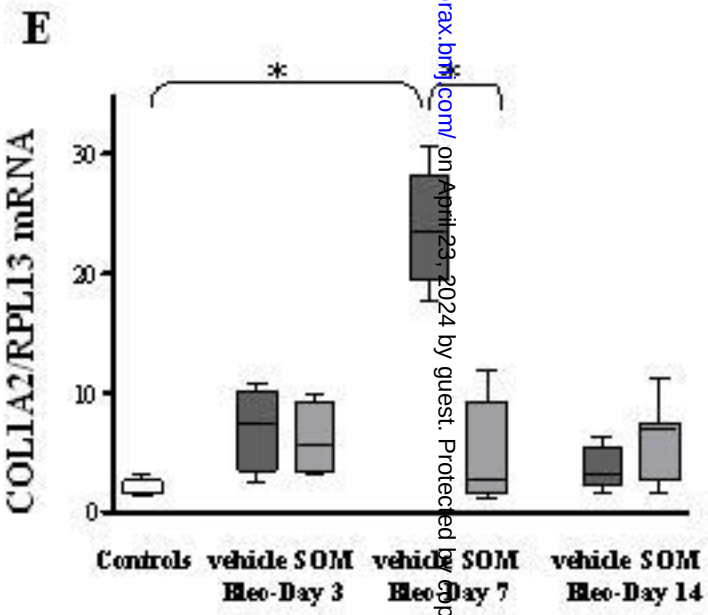


Figure 5

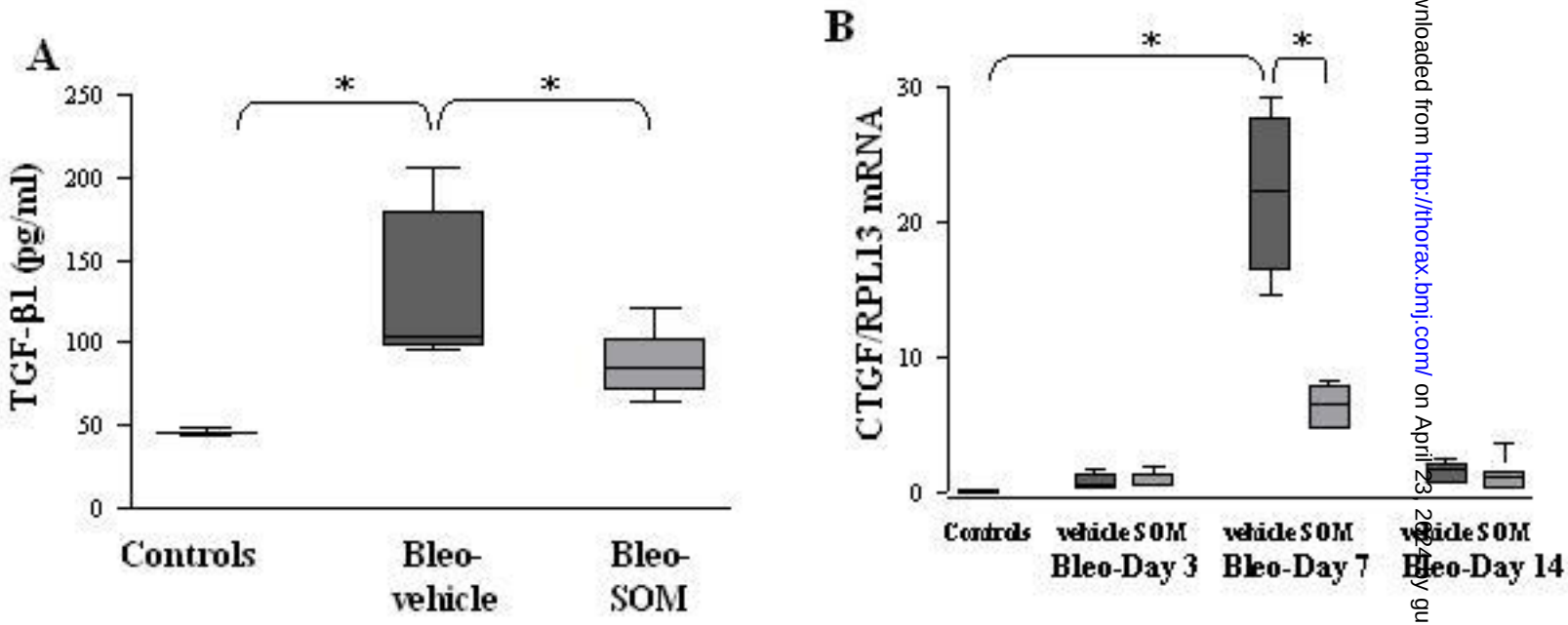


Figure 6

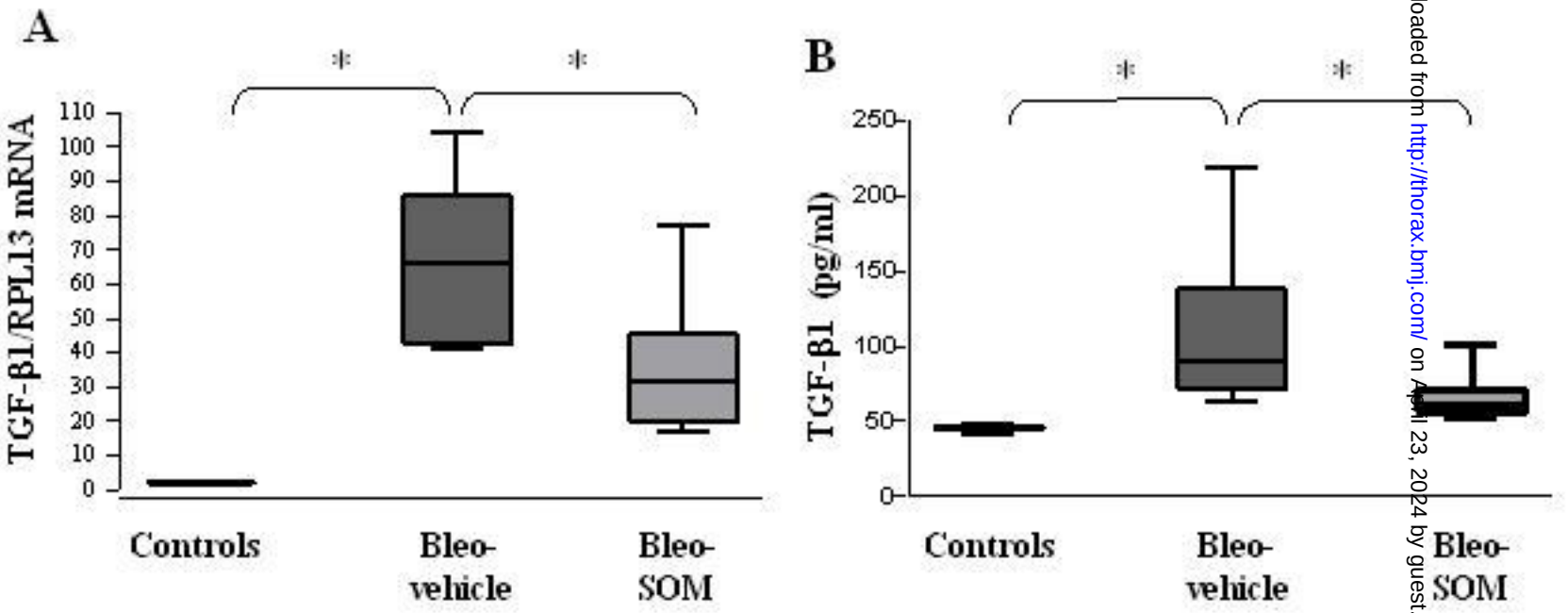


Figure 7