Changes in xylosyltransferase activity and in proteoglycan deposition in bleomycin-induced lung injury in rat

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Changes in xylosyltransferase activity and in proteoglycan deposition in bleomycin-induced lung injury in rat. R. Koslowski, U. Pfeil, H. Fehrenbach, M. Kasper, E. Skutelsky, K-W. Wenzel. ©ERS Journals Ltd 2001.
ABSTRACT: Several lines of evidence support the hypothesis of the involvement of altered proteoglycan deposition in the development of lung diseases. UDP-D-xylose: core protein β-D-xylosyltransferase (UDP-xylosyltransferase; EC 2.4.2.26) is a key enzyme for the glycosylation of proteoglycan core proteins. This study examined the catalytic activity of UDP-xylosyltransferase in lung tissue and in isolated fibroblasts, as well as the deposition of the proteoglycans versican, biglycan and decorin in rat lung tissue during bleomycin-induced lung injury.

Rats were given, endotracheally, a single dose of bleomycin. Deposition of proteoglycans in lung tissue was assessed by immunohistochemistry and the catalytic activity of xylosyltransferase was determined with an acceptor peptide of the sequence Q-E-E-E-G-S-G-G-G-Q-G-G as a substrate.

The results show coincidence of increasing xylosyltransferase activities in lung tissue with accumulation of versican at alveolar entrance rings and in fibrotic regions in close proximity to α-smooth muscle actin-positive cells. In contrast, no changes in biglycan and decorin deposition in fibrotic lungs were observed, except for decorin in alveolar type II pneumocytes and alveolar macrophages. Bleomycin treatment of isolated rat lung fibroblasts resulted in a concentration-dependent increase of xylosyltransferase activity up to 2 mU bleomycin·mL^{-1}.

The data suggest a participation of myofibroblasts with increased xylosyltransferase activities in accumulation of versican in fibrotic foci of injured lung tissue at the early stages of development of lung fibrosis.
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Pulmonary fibrosis develops in response to lung injuries caused by either radiation, chemical agents or pulmonary infection. It is characterized by alterations of alveolar epithelium, proliferation of fibroblasts, accumulation of components of the extracellular matrix and thickening of the alveolar walls [1–3]. Pulmonary fibrosis is the major side-effect of the antineoplastic agent bleomycin. Intratracheal administration of bleomycin into the lung of rats is frequently used for induction of experimental lung fibrosis [1, 3–12].

Proteoglycans consisting of glycosaminoglycan chains covalently attached to a core protein are major components of the nonfibrillar part of the extracellular matrix filling the space between the fibrous proteins. They modulate cellular adhesion, proliferation and cell migration [13]. In the distal parts of the lung, proteoglycans are important for the structural integrity of the alveolar wall [14, 15]. In the alveolar interstitium, fibroblasts are the predominant source of proteoglycans [16–18].

There are several lines of evidence suggesting involvement of altered proteoglycan deposition in the development of lung diseases, such as adult respiratory distress syndrome [17, 19], sarcoidosis [20], bronchiolitis obliterans [17, 21], extrinsic allergic alveolitis [20], tuberculosis [20], or idiopathic pulmonary fibrosis [20, 22]. In bleomycin-induced lung injury in the rat or hamster, accumulation of hyaluronan was described as preceding pulmonary fibrosis [4, 5, 23, 24].

The small proteoglycans decorin and biglycan, which contain chondroitin sulphate or dermatansulphate chains, bind to dermal elastic fibres [25]. Decorin is found closely associated with collagen in the lung [26] and it may play a role in the regulation of collagen fibrillogenesis [27, 28]. Both decorin and biglycan are able to bind transforming growth factor (TGF)-β and to inactivate it [29–32], giving rise to the hope of using decorin to decrease excessive levels of free TGF-β observed in lung fibrosis [11]. WESTERGREN-THORSSON et al. [33] reported increased messenger ribonucleic acid (mRNA) levels and proteoglycan amounts for biglycan, and an opposite pattern for decorin in bleomycin-induced fibrosis in rats. It was suggested that versican, a large chondroitin sulphate proteoglycan (CSPG), forms a provisional matrix for collagen deposition in areas of developing pulmonary fibrosis [17, 20].

An important step for the formation of most of the

polysaccharide side chains of connective tissue proteoglycans, is the transfer of D-xylose from UDP-xylose to certain serine residues of proteoglycan core proteins by UDP-D-xylose: core protein β-D-xylosyltransferase (UDP-xylosyltransferase; EC 2.4.2.2.6). This enzyme initiates a series of post-translational modifications necessary for transport and secretion of proteoglycans [34].

This study describes the localization and increased deposition of chondroitin sulphate proteoglycans in the alveolar region of bleomycin-induced lung injury. The time course of changes in the proteoglycan deposition and in the activity of xylosyltransferase in lung tissue and in lung fibroblasts was investigated.

Materials and methods

Animals, bleomycin administration and tissue preparation

Female rats of a Wistar strain, weighing ∼200 g, received under ketamine anesthesia, a single dose of 7.5 units bleomycin sulphate·kg^{-1} body weight (Mack, Illertissen, Germany) dissolved in 0.25 mL of 0.9% NaCl solution, by endotracheal instillation as described previously [12]. Controls received an equal volume of the vehicle. The animals were sacrificed at given times and the lungs were flushed free of blood across the right ventricle with phosphate-buffered saline (PBS), pH 7.4, containing 50 U heparin·mL^{-1}.

Lung tissue was minced in two volumes of 100 mM Tris/HCl, pH 7.0, containing 250 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 5 mM benzamidine hydrochloride, 2 mM iodoacetic acid and 1 μM soybean trypsin inhibitor. After homogenization with a motor driven teflon pestle, the homogenate was centrifuged at 100,000×g at 4°C for 1 h. The supernatant was dialysed against 50 mM Tris/HCl, pH 7.0, containing 50 mM NaCl.

For immunohistochemical studies, lungs were fixed by immersion in 4% phosphate-buffered formalin and embedded in paraffin as described earlier [35]. The time course of bleomycin-induced changes in CSPG and versican distribution was followed by examination of lungs fixed 7, 14, 21, 28, 35, or 42 days after treatment (n=2). Control lungs were obtained from two untreated animals. To evaluate bleomycin-induced fibrosis, sections of lung tissue were stained with polyclonal rabbit antirat collagen I (Chemicon, Temecula, CA, USA).

Preparation of lung fibroblasts and bleomycin treatment

Fibroblasts from the lungs of 3-month-old female Wistar rats were prepared by enzymic dissociation of lung tissue after vascular perfusion and repeated bronchoalveolar lavage with PBS using 0.2% collagenase type I (Sigma, Deisenhofen, Germany), 0.15% pronase (Boehringer, Mannheim), 0.08% dispase (Boehringer) and 0.9% DNase (Sigma). The isolated cells were filtered (filter holes 100 μm), collected by centrifugation and resuspended in Roswell Park Memorial Institute (RPMI) 1640 culture medium supplemented with 10% foetal calf serum (Biochrom, Berlin, Germany), 50 IU penicillin·mL^{-1} and 50 μg streptomycin·mL^{-1}. Cells were passaged routinely and the media were changed every 3 days. Cells between passage numbers 4 and 20 were used for experiments.

For bleomycin treatment, subconfluent lung fibroblasts were deprived of serum for 24 h and treated with bleomycin for 1 h. The medium was then changed, the cells were kept free of serum for 12 h and harvested using 0.05% trypsin with 0.5% mM EDTA (Biochrom). Viability was checked by trypan blue exclusion.

For preparation of homogenates, cells were suspended in 100 mM Tris/HCl, pH 7.0, containing 250 mM NaCl, 1 mM EDTA, 5 mM benzamidine hydrochloride, 2 mM iodoacetic acid, 1 μM soybean trypsin inhibitor and 0.5% Triton X-100 with brief sonication. After incubation for 12 h, samples were centrifuged at 100,000×g at 4°C for 1 h, and the supernatant was dialysed against 50 mM Tris/HCl, pH 7.0 containing 50 mM NaCl for 12 h.

Determination of UDP-xylosyltransferase activity

UDP-xylosyltransferase activity was determined as described previously [34]. Briefly, the reaction mixture contained in a final volume of 100 μL: 320 μM acceptor peptide of the sequence Q-E-E-E-G-S-G-G-G-Q-G-G, 0.46 μM UDP-[^{14}C]-D-xylose, 68 μM UDP-D-xylose, 5 mM $MnCl_2$ and cell or tissue homogenates. After incubation at 37°C for 1 h, protein was precipitated by addition of 0.5 mL 10% trichloroacetic acid/4% phosphotungstic acid, washed and redissolved in 1 M NaOH for liquid scintillation counting. Xylosyltransferase activity was calculated from the difference of UDP-D-xylose initially employed and D-xylose bound to the acceptor peptide. One mU of enzymic activity represents the incorporation of 1 nmol xylose·min^{-1} into the acceptor peptide. The acceptor peptide was obtained by solid-phase peptide synthesis (9050 PepSynthesizer, MilliGen/Biosearch, Bedford, MA, USA). Protein concentrations were determined according to BRADFORD [36].

Immunoperoxidase staining of proteoglycans

For immunohistochemical localization of CSPGs, rabbit antichondroitin sulphate proteoglycan antiserum (Bioproducts, Ingelheim, Germany) was used. This antiserum reacts with proteoglycans containing glucuronic acid-N-acetyl-galactosamine (GlcU-GalNAc) units unmasked by chondroitinase ABC digestion. Versican was detected using anti-full-length human versican antiserum (kindly provided by E. Ruoslahti, Cancer Research Center, La Jolla, CA, USA [37]). The antisera LF-106 against synthetic mouse biglycan peptide and LF-113 against synthetic mouse decorin peptide were the kind gift of L.W. Fisher, NIH, Bethesda, MD, USA [38]. Antisera LF-106 and LF-113 are immunoreactive with rat proteoglycans and do not cross-react with each other [39, 40].

Paraffin sections were mounted on silane coated slides, dried for 12 h, and dewaxed. Unless stated otherwise, the following steps were performed at 25°C. Sections to be stained for biglycan and decorin were processed without pretreatment. Tissue sections to be stained for CSPG were microwave-irradiated at 750 W (twice for 5 min each) in 10 mM sodium citrate, pH 6.0. The sections were then incubated in 50 mM Tris/HCl, pH 8.0, containing 50 mM NaCl and 0.5 µL chondroitinase ABC·mL^{-1} from *Proteus vulgaris* (Sigma), at 37°C for 90 min. Dewaxed tissue sections to be stained for versican, and collagen type I were pretreated with 0.1% pronase (Merck, Darmstadt, Germany) in PBS for 15 min. After blocking of endogenous peroxidase and unspecific antibody binding, tissue sections were incubated with antisera against either CSPG (dilution 1:50), versican (dilution 1:50), decorin (dilution 1:100), biglycan (dilution 1:100) or collagen I (dilution 1:50, all dilutions in PBS) at 37°C for 1 h. For detection of primary antibodies, tissue sections were washed with PBS for 10 min and incubated with peroxidase-coupled goat antirabbit immunoglobulin-G (IgG) (horse radish peroxidase (HRP)77; H. Grossmann, Dresden, dilution 1:400 in PBS containing 50% foetal calf serum) for 60 min, followed by incubation with 1.67 mM diaminobenzidine for 8 min. The ABC Vectastain kit (Vector Laboratories Burlingame, CA, USA) was used to visualize the binding of the monoclonal anticollagen I antibody. After rinsing in PBS, counterstaining with haematoxylin was performed. To reduce background staining in the immunohistochemical localization of versican, PBS was supplemented with 0.1% acetylated bovine serum albumin (Aurion, Köln, Germany). For control of specificity, primary antibodies were replaced by PBS.

Detection of proteoglycans with cationic colloidal gold

Cationic colloidal gold (CCG) of 12 nm average diameter was stabilized with poly-L-lysine of 27 kDa molecular mass [41]. For ultrastructural localization of polyanionic proteoglycans, pieces of normal and fibrotic lung tissue were fixed in 0.1 M sodium cacodylate containing 4% formaldehyde and 0.2% glutaraldehyde [41, 42]. As described earlier [43], the lungs were infiltrated with PBS containing 2.3 M sucrose, frozen in liquid nitrogen, cryo-substituted with methanol at -80°C, embedded in Lowycryl HM20 and polymerized at -30°C in a Reichert AFS (Leica, Hamburg, Germany). Ultrathin sections were mounted on 200 mesh nickel grids precoated with 3% colloidin. Sections from fibrotic lungs were incubated in 100 mM Tris/HCl, pH 7.0, containing 137 mM NaCl, 40 mM CaCl$_2$ and 1 U of heparinase III·mL^{-1} from *Flavobacterium heparinum* or in 50 mM Tris/HCl, pH 8.0, containing 50 mM NaCl and 1 U chondroitinase ABC·mL^{-1} from *Proteus vulgaris* (Sigma), at 37°C for 2 h and washed thoroughly with PBS. Control sections were incubated with PBS containing 1% bovine serum albumin for 5 min. All sections were washed with PBS, labelled with CCG (dilution 1:40 in PBS) for 1 h, jet-washed with distilled
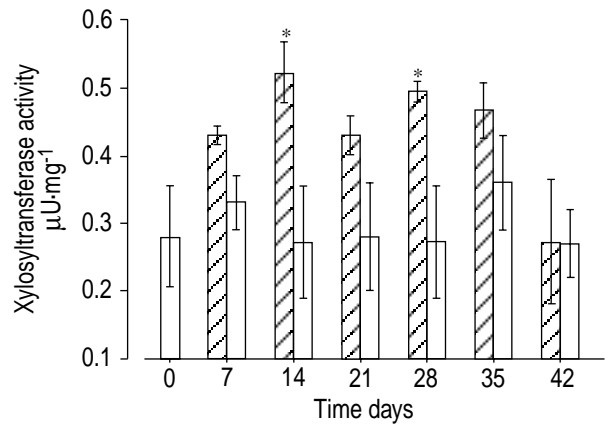

Fig. 1. – Changes in the specific activity of UDP-D-xylose: core protein β-D-xylosyltransferase (UDP-xylosyltransferase) in lung tissue following intratracheal administration of bleomycin (mean±SD, n=5). □: lung tissue of controls; ▨: lung tissue of bleomycin-treated rats. *: p<0.05, treated group *versus* controls, Kruskal-Wallis H-Test.

water, and counterstained with saturated uranyl acetate in 50% ethanol for 30 min.

Results

Activities of UDP-xylosyltransferase

Figure 1 shows the time course of changes in the specific activity of UDP-xylosyltransferase in rat lung tissue following intratracheal bleomycin administration. The activity of the enzyme increased by ~1.6-fold 7 days after bleomycin treatment and reached a maximum of ~2-fold of controls at day 14. Xylosyltransferase activity remained at a high level up to 35 days post-bleomycin administration. Thereafter, the level declined and reached control values at day 42.

Treatment of isolated rat lung fibroblasts with bleomycin for 13 h resulted in increasing xylosyltransferase activities (fig. 2). The increase of the activity seems to depend on bleomycin concentration,
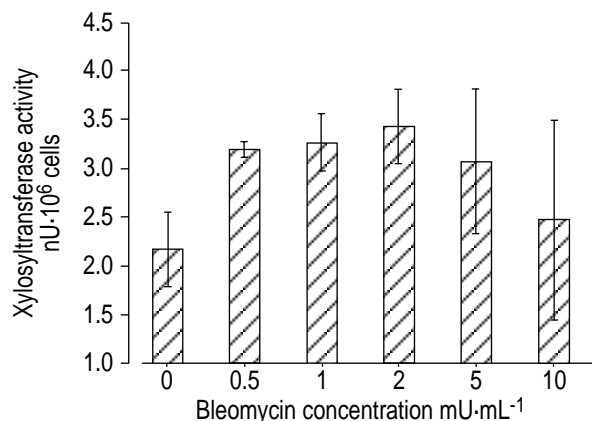

Fig. 2. – Specific activity of UDP-D-xylose: core protein β-D-xylosyltransferase (UDP-xylosyltransferase) in isolated rat lung fibroblasts treated with bleomycin for 13 h (mean±SD, n=3).

however, the differences were not significant. The maximum value of ~1.6-fold of controls was observed at 2 mU bleomycin·mL^{-1}. At higher concentrations of bleomycin the UDP-xylosyltransferase activity was found at lower levels. Toxic effects of the cytostatic drug may interfere with the stimulatory effect on xylosyltransferase activity.

Immunohistochemical localization of proteoglycans

CSPG and the individual proteoglycans, versican, biglycan and decorin exhibited distinct distribution patterns in the normal rat lung (table 1). Anti-CSPG immunoreactivity was exclusively extracellular. Staining for CSPG was most prominent in the peribronchial (lamina propria, submucosa) and perivascular (adventitial) interstitium. Except for alveolar entrance rings, alveolar septae exhibited only weak staining of the extracellular matrix (fig. 3a). Anti-versican immunoreaction was restricted to the lamina propria beneath the bronchiolar epithelium and the vascular endothelium, and to the tunica media of vessels, in particular to internal and, where present, external elastic lamina. Only traces of versican were detected in the interstitium of alveolar walls, even at alveolar entrance rings (fig. 3b). Decorin was detected in the connective tissue of the vascular adventitia, whereas for biglycan most prominent staining was associated with the tunica media of pulmonary vessels. Both proteoglycans were detected in the airway epithelium (table 1). There was no staining of any parenchymal component for decorin or biglycan (fig. 3c). Intense immunoreactivity for collagen I occurred in the vascular tunica adventitia and in the lamina propria (fig. 4a, b). Furthermore, the submucosa of airways was stained (fig. 4b), whereas the alveolar parenchyma was lacking of collagen I immunoreactivity (fig. 4c).

In bleomycin-treated rat lungs, CSPG increased with time after treatment (fig. 3d, f, i). This was also accompanied by increasing versican deposition (fig. 3e, g, j). As early as 7 days after treatment, an increase in the parenchymal immunoreactivity for CSPG and for versican, respectively, was observed, particularly at alveolar entrance rings.

The increasing deposition of both CSPG and versican was closely associated with the development of fibrotic alterations at alveolar entrance rings (fig. 3d, e) and an increase in the number of α-smooth muscle actin-positive cells at sites of fibrotic lesions was observed. Fibrotic plugs showed prominent staining of the extracellular matrix in proximity to α-smooth muscle actin-positive cells for both CSPG and versican (fig. 3j). In contrast, biglycan and decorin did not show any fibrosis-relevant changes, with the exception of the appearance of a weak intracellular staining of alveolar type II pneumocytes and macrophages for decorin (fig. 3h). Deposition of collagen I was observed in the alveolar interstitium at alveolar entrance rings (fig. 4d) and in regions showing thickened alveolar walls (fig. 4e).

Detection of polyanionic components with cationic colloidal gold

In normal lung tissue, polyanionic components were localized at the basal lamina of the alveolar epithelium, whereas weak labelling was associated with collagen fibrils (fig. 5a). Twenty-eight days after bleomycin treatment, increased labelling of the extracellular matrix with CCG was detected in fibrotic areas of lung tissue, particularly in association with bundles of collagen and elastin fibrils (fig. 5b). To elucidate, if the polyanions associated with fibrous extracellular matrix components are proteoglycans belonging to CSPGs or heparansulphate proteoglycans (HSPGs), ultrathin sections of fibrotic lung tissue were treated with chondroitinase ABC or heparinase III. No CCG labelling of the collagen bundles was observed after heparinase treatment (fig. 5c, d).

Table 1. – Main sites of immunohistochemical staining for proteoglycans and collagens in normal and fibrotic rat lungs

Region	CSPG	Versican	Biglycan	Decorin	Collagen I
Normal rat lung					
Airways					
Epithelium	-	-	+	+	-
Lamina propria	++	++	-	-	++
Submucosa	++	-	-	+	++
Vessels					
Endothelium	-	-	-	-	-
Lamina propria	+	++	-	+	+
Media	+	+	++	-	+
Adventitia	++	-	+	++	++
Alveolar walls					
Extracellular	++	+	-	-	+
Intracellular	-	-	-	-	-
Changes associated with bleomycin-induced pulmonary fibrosis in rat	Increased extracellular staining in fibrotic foci	Increased extracellular staining in fibrotic foci	Weak intracellular staining (PII, AM)	Weak intracellular staining (PII, AM)	Increased extracellular staining

-: no staining; +: weak staining; ++: prominent staining; CSPG: Chondroitinsulphate proteoglycan; PII: alveolar type II pneumocytes; AM: alveolar macrophages.
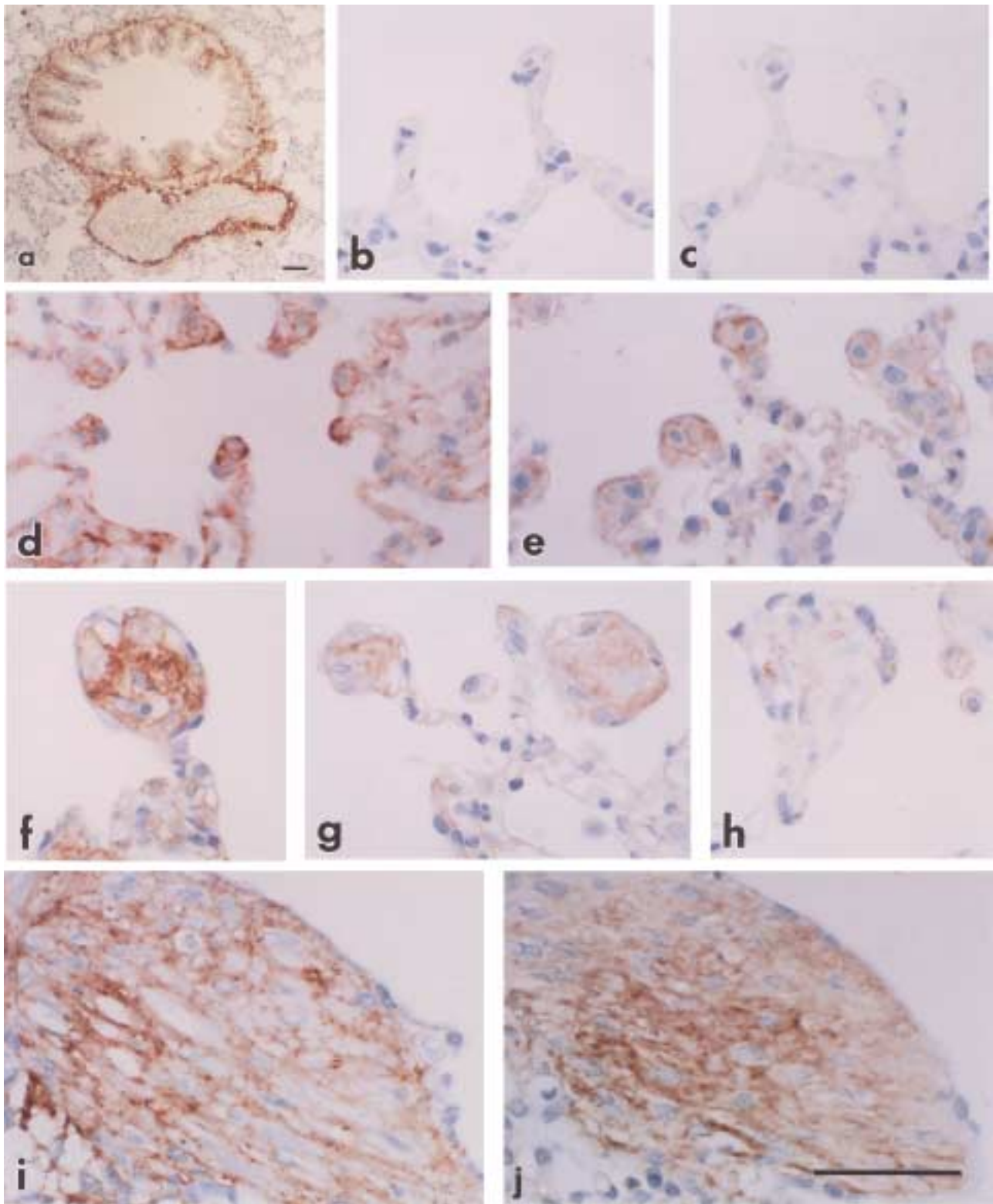
Fig. 3. – Localization of proteoglycans in normal (a–c) and in fibrotic (d–j) rat lung. Chondroitinsulphate proteoglycan (CSPG) (a, d, f, i), versican (b, e, g, j) and decorin (c, h) at days 7 (d, e), 14 (f–h) and 28 (i, j) following bleomycin administration. Note the staining for CSPG at alveolar entrance rings in normal lung tissue (a), whereas no versican (b) or decorin (c) was detected in alveolar parenchyma. Increased amounts of CSPG (a, d, f, i) and versican (b, e, g, j), respectively, were found in the extracellular matrix. For decorin, an increase in intracellular staining was detected (c, h). Scale bars=50 μm.
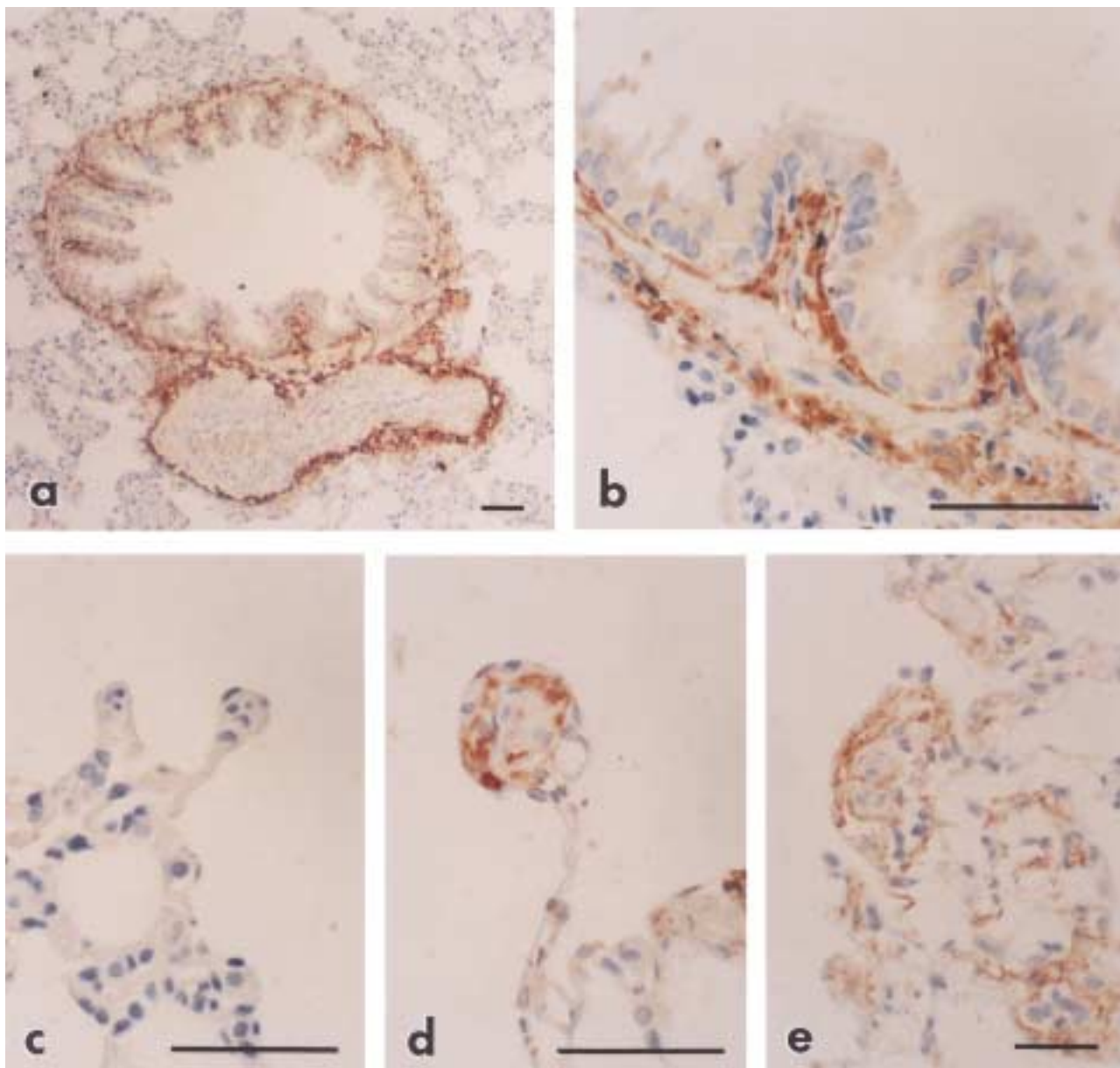
Fig. 4. – Localization of collagen I in normal (a–c) and fibrotic (d, e) rat lung tissue at day 35 following intratracheal bleomycin administration. In normal rat lung, intense staining is associated with vascular tunica adventitia and lamina propria as well as submucosa of airways (a, b), whereas parenchyma shows no collagen I immunoreactivity (c). Bleomycin treatment resulted in an increase of collagen I at alveolar entrance rings and in fibrotic foci (d, e). Scale bars=50 μm.

Treatment with chondroitinase ABC did not result in any reduction of CCG labelling of collagen fibrils (data not shown).

Discussion

The results presented in this study demonstrate coincidence of increasing catalytic activities of UDP-xylosyltransferase with increasing deposition of CSPG in general, and of versican as an individual CSPG in fibrotic regions of rat lungs following intratracheal bleomycin administration.

Proteoglycans consist of a core protein and glycosaminoglycan side chains. UDP-xylosyltransferase catalyzes the transfer of D-xylose from UDP-D-xylose to certain serine residues in the core proteins of proteoglycans as a rate-limiting step for glycosylation. There have been several potential attachment sites for glycosaminoglycan side chains reported for the versican core protein [13, 37]. The assembly of the entire molecule depends on the synthesis of the core protein and on the velocity of the glycosylation.

The increased immunoreactivity for versican observed at alveolar entrance rings and in fibrotic foci in bleomycin-injured rat lungs may be a sign for elevated core protein synthesis regardless of glycosylation, because the antiserum against versican
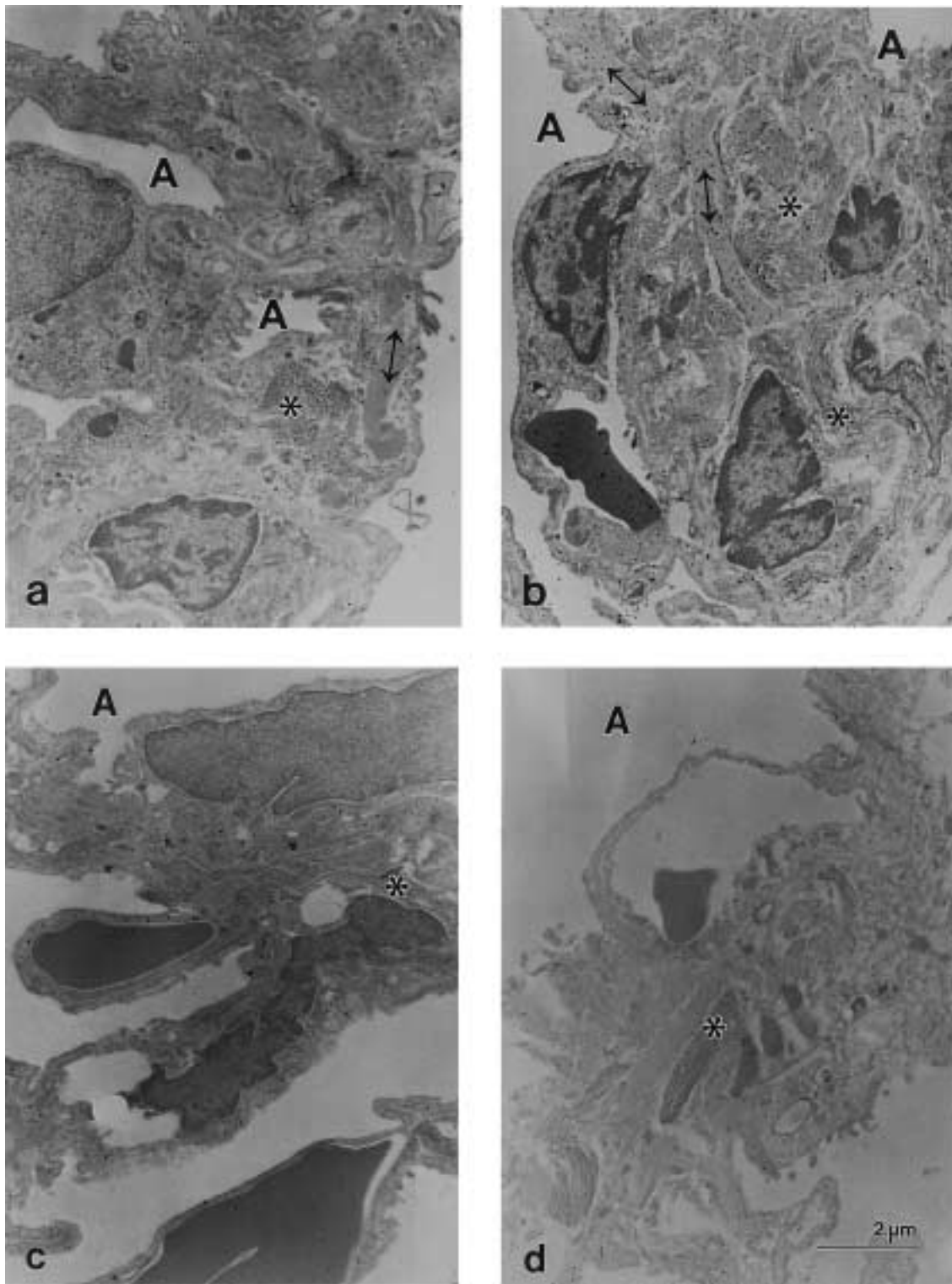
Fig. 5. – Localization of polyanionic compounds in normal (a) and fibrotic rat lung at day 28 following intratracheal bleomycin administration (b–d). Polyanions were detected by cationic gold labelling and evaluated by electron microscopy. In normal lung (a) and normal appearing lung tissue of bleomycin-treated lung (c), cationic colloidal gold (CCG) was predominantly associated with basal membranes of the air blood-barrier. In fibrotic foci of the bleomycin-treated lung (b), an increase in CCG associated with collagen bundles (*) and elastic fibres (double-headed arrows) was observed, which was abolished by pretreatment of ultrathin sections with heparinase III (d). A: air space. Scale bar=2 μm.

recognizes the core protein. Anti-CSPG antiserum reacts with GlcU-GalNAc units unmasked by chondroitinase ABC digestion and, therefore, detects all CSPGs. The colocation of CSPG and versican observed in fibrotic areas of injured lung tissue indicates that a considerable fraction of the CSPG detected in these regions represents glycosylated versican, although the number of glycosaminoglycan side chains is unknown. Increased glycosylation of the core protein alone does not explain the increase in immunoreactivity for versican. At minimum, a coordinated regulation of both core protein synthesis and xylosyltransferase activity ensures the accumulation of the entire proteoglycan. After bleomycin administration, elevated catalytic activies of xylosyltransferase were found in injured lung tissue, accompanied by increased deposition of CSPG and versican. Bleomycin treatment of isolated lung fibroblasts resulted in concentration-dependent changes in xylosyltransferase activity. At low bleomycin concentrations, enzymic activity increased slightly, whereas at bleomycin concentrations >5 $mU \cdot mL^{-1}$, xylosyltransferase activity was found decreased, probably due to toxic effects of bleomycin on the cells. UDP-xylosyltransferase was recently purified from rat ear cartilage and some properties were studied [34]. So far, nothing is known about the regulation of the biosynthesis of this transferase. KÄHÄRI et al. [44] reported that in human fibroblasts TGF-β_1 upregulates versican and biglycan mRNA synthesis, and downregulates decorin mRNA synthesis [44]. LIU et al. [45] reported on platelet-derived growth factor (PDGF)-BB-induced stimulation of sulphate incorporation into biglycan without affecting the biglycan core protein mRNA level. TGF-β and PDGF seem to be candidates involved in the regulation of xylosyltransferase.

Accumulation of versican starts in the interstitium of alveolar entrance rings. In the alveolar septa, myofibroblasts sharing morphologic and biochemical features of both fibroblasts and smooth muscle cells are prominent [46, 47]. At the tips of alveolar septa, a smooth muscle cell-type predominates. α-Smooth muscle actin is a reliable differentiation marker of myofibroblasts, but it does not allow differentiation between myofibroblasts and smooth muscle cells. Rat alveolar fibroblasts acquire α-smooth muscle actin as early as 24 h after intratracheal instillation of bleomycin, and these myofibroblasts are the predominant cells at sites of active fibrosis in the bleomycin-injured lung where they synthesize $\alpha_1(I)$ procollagen [9, 10, 44, 47–49]. The present data suggest that the versican deposition at the septal tips is a result of increased synthesis by smooth muscle cells, as was shown by WIGHT et al. [13], whereas myofibroblast-like cells represent the source of versican in fibrotic foci. Close association of versican and collagen I with myofibroblasts in the extracellular matrix of fibrotic plugs of rat lung, as shown here, confirms the data presented by BENSADOUN et al. [17] on accumulation of versican in early fibrotic foci of idiopathic pulmonary fibrosis and in granulomatous lung diseases in humans [20]. The deposition of versican precedes the accumulation of collagen in the alveolar interstitium, suggesting versican is a constituent of a provisional matrix influencing functions of myofibroblasts embedded in this matrix [17, 20].

The present results are partly in contrast to the report of WESTERGREN-THORSSON et al. [33] who described increased biglycan levels and decreased decorin levels in bleomycin-induced lung fibrosis in rat. However, in their study [33], mRNA levels and proteoglycan amounts were determined after extraction and purification of proteoglycans. VENESS-MEEHAN and coworkers [50, 51] reported on immunohistochemical detection of biglycan and decorin in lungs of newborn Sprague-Dawley rats exposed to hyperoxia. The present study's results confirm the reports on decorin core protein detection surrounding blood vessels and immunoreactivity for both biglycan and decorin in association with airways [50, 51]. In 6-week-old control animals, VENESS-MEEHAN et al. [50] showed an overall decrease of immunostaining for biglycan. The present study used 3-month-old Wistar rats and the difference in age, and probably in rat strain, may explain the lack of staining of alveolar epithelium and of alveolar walls in the present study.

Taken together, the data suggest that the deposition of versican is an early event throughout the development of bleomycin-induced pulmonary fibrosis, followed by accumulation of collagen at the same location where myofibroblasts predominate. The coincidence of the increase of UDP-D-xylose: core protein β-D-xylosyltransferase activity with the accumulation of versican supports the hypothesis of a coordinated regulation of the synthesis of both xylosyltransferase and proteoglycans.

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