Regulation of Kininogen Gene Expression and Localization in the Lung after Monocrotaline-Induced Pulmonary Hypertension in Rats (43597)

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Abstract. **Pyrrolizidine monocrotaline (MCT) from plant seed produces pulmonary endothelial cell injury, pulmonary hypertension, and inflammation in rats, providing a useful animal model for studying progressive pulmonary vascular disease. Kininogen is the precursor of proinflammatory kinins and may also exert anti-inflammatory actions by inhibiting cysteine proteinases. Given the potential roles of kininogen in vascular injury and inflammation, we have investigated the regulation of kininogen gene expression in the MCT-induced pulmonary hypertensive rat model. Sprague-Dawley rats, in groups of six, were given a single subcutaneous injection of monocrotatine (60 mg/kg body wt) and sacrificed 10 and 20 days later. Northern blot hybridization using a kininogen cDNA probe showed kininogen gene expression in the liver, lung, and kidney. MCT treatment induced a time-dependent increase in kininogen mRNA levels, whereas it reduced rat α_1-antitrypsin and kallikrein-binding protein mRNA levels in the liver. Similarly, kininogen mRNA levels were low in the normal lung and were increased 7.5- and 13.7-fold, respectively, after MCT injection for 10 and 20 days. Immunoreactive kininogen levels in perfused liver and lung extracts of rats receiving MCT injection increased up to 20-fold, as measured by a T-kininogen radioimmunoassay. Western blot analyses showed that a 68-kilodalton immunoreactive kininogen increased in the serum and lung extracts of MCT-treated rats compared to those in the control rats. In control rats, immunostaining for kininogen in the lung was most marked in venous endothelial cells and alveolar macrophages. After MCT treatment, staining for kininogen increased dramatically throughout the lung tissues, often covering the epithelial surfaces of alveoli and bronchi. The present studies have shown that the toxin MCT altered the synthesis and distribution of pulmonary kininogen and suggest that the kininogen/kinin system may be associated with the pulmonary vascular injury, remodeling, and inflammation seen in this animal model.** [P.S.E.B.M. 1993, Vol 203]

Monocrotaline (MCT), a pyrrolizidine alkaloid from plant seed (*Crotalaria spectabilis*), causes lung injury, with pulmonary edema, inflammation, and progressive pulmonary hypertension, in rats (1–3). Time-course studies indicate that the lung injury and edema occur relatively early after MCT administration, whereas the progressive hypertensive pulmonary vascular injury, remodeling, and pulmonary hypertension become apparent later (4). Since the pathology of hypertension and inflammation in rats mimics symptoms seen in humans, the MCT-induced pulmonary hypertension in rats serves as a useful animal model for chronic pulmonary vascular diseases in humans.

The mode of action of MCT in producing pulmonary hypertensive disorders is not clear. Many studies have documented altered levels of potential mediators of lung injury and inflammation, such as interleukin-1 and polyamines (5, 6). Further, angiotensin-converting enzyme activity in the lung, but not in the serum, is

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significantly reduced in rats with pulmonary hypertension produced by subcutaneous injection of monocrotaline (7). Altered elastase and collagen synthesis have been shown to be associated with MCT-induced vascular alteration and pulmonary hypertension (8). The fate of vasoactive autacoids in the pulmonary circulation has also been shown to be altered after MCT-induced lung vascular injury in rats (9). In addition, structural alterations in the pulmonary vascular endothelium have been observed in both experimental models of pulmonary hypertension (10) and clinical studies (11). Given the role of the kininogen-kinin system in vascular homeostasis and inflammation, we investigated the potential association of the kininogen-kinin system with MCT-induced pulmonary injury.

Kininogens, which have multifunctional domains, serve as kinin precursors, cysteine proteinase inhibitors, blood coagulation cofactors, and acute phase proteins (12). Kininogens are potent cysteine proteinase inhibitors and may enhance wound healing by inhibiting lysosomal proteinases and, therefore, play a protective role in tissue injury during acute phase inflammation. Additionally, they may act as proinflammatory agents, as they are precursors of kinin peptides. There are three distinct forms of kininogen: (i) high molecular weight (HMW) kininogen; (ii) low molecular weight (LMW) kininogen (K-kininogen), both transcribed from a single gene (13); and (iii) T-kininogen (another LMW kininogen), which has been identified as an acute phase protein in the rat (14). Kinin peptides have a broad spectrum of biological activities, including vasodilatation, vasoconstriction, increased vascular permeability, smooth muscle contraction, ion transport, cell proliferation, pain production, and inflammation (15). The kininogen-kinin system has been linked to several pathophysiological conditions, including secondary vascular changes in experimental brain injury (16), and recent studies in a rat spinal cord injury model showed increased accumulation of kininogen and its conversion to proinflammatory kinins in the traumatized tissues (17).

To further understand the regulation and function of kininogens *in vivo*, we have purified kininogens from various species (18), developed specific polyclonal and monoclonal antibodies (19, 20), and isolated cDNA clones encoding rat and human kininogens from cDNA libraries (21). These protein and cDNA reagents have been used as probes to study the regulation of kininogen gene expression by hormones and drugs (22). In the present studies we show by Northern blotting, Western blotting, direct radioimmunoassay (RIA), and immunohistochemistry, that MCT administration induces kininogen gene expression in the liver and lung, and results in tissue redistribution of pulmonary kininogen.

Materials and Methods

Animal Treatment and Tissue Extract Preparation. Sprague-Dawley rats (male, 200–250 g body wt), in groups of six were injected subcutaneously with phosphate-buffered saline (control) or a single dose of MCT (60 mg/kg). After 10 or 20 days, the rats were anesthetized with pentabarbitol (50 mg/kg). The heart was exposed, and 5 ml of blood were withdrawn. Heparin (100 units/rat) was injected into the left ventricle. After 30 sec, the vena cava was cut, and the circulation was perfused via cardiac puncture through the left ventricle with at least 30 ml of normal saline until tissues appeared blood free. Perfused tissues were used for the protein assay, RNA extraction, and immunohistochemical studies. Tissues were removed, minced, and homogenized in phosphate-buffered saline (pH 7.3) at 4°C. Homogenates were centrifuged at 600*g* for 20 min at 4°C, and the supernatant was treated with deoxycholate (0.5%, w/v) for 30 min at room temperature. The supernatants were collected after centrifugation at 20,000*g* for 30 min at 4°C, and protein concentrations were determined by the method of Lowry *et al.* (23), using bovine serum albumin as the standard.

Kininogen RIA. Kininogen levels were measured by a direct RIA, as described previously (20). Briefly, purified T-kininogen (5 μg) was labeled with ^{125}I using a lactoperoxidase method, and the labeled T-kininogen was separated from the reaction mixture with a Sephadex G-100 column. In the antibody titration curve, T-kininogen antiserum dilutions in the assay buffer ranged from 1:1,000 to 1:640,000. One hundred microliters of ^{125}I-labeled T-kininogen (10,000 cpm/100 μl) and 100 μl of antibody in the assay buffer were added to 200 μl of assay buffer, bringing the final volume to 400 μl. The assay mixtures were incubated at room temperature for 18 hr. Antibody-bound T-kininogen was separated from free T-kininogen through centrifugation in an optimum combination of polyethylene glycol and bovine γ-globulin. The final antiserum dilution was 1:480,000, and the standard ranged from 160 pg to 20 ng.

Western Blot Analysis. Aliquots of rat serum (1 μl) or lung tissue extract (50 μg of protein) were mixed with an equal volume of extraction buffer containing 0.125 *M* Tris-HCl (pH 6.8), 30% (v/v) glycerol, 5% sodium dodecyl sulfate (SDS), and 40 m*M* dithiothreitol, and the mixture was heated to 100°C for 5 min before electrophoresis. SDS-polyacrylamide gel electrophoresis was performed in a 7.5–15% gradient gel in Tris-glycine buffer, pH 8.8. Proteins were then electrophoretically transferred onto nitrocellulose membrane using a Trans-Blot cell (Bio-Rad, Richmond, CA). The immunoblotting procedures were similar to those described previously using rabbit antirat T-kininogen

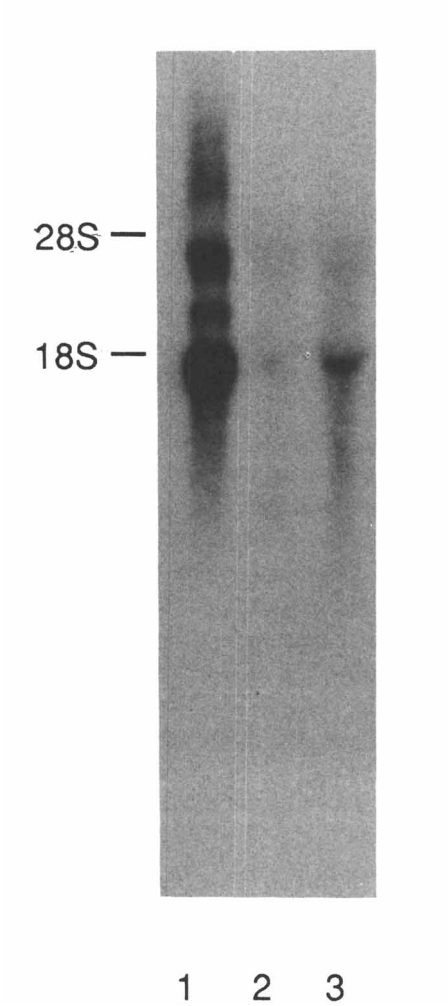

Figure 1. Northern blot analysis of kininogen mRNA in normal liver, kidney, and lung using a LMW K-kininogen cDNA probe. (Lane 1) Liver RNA (10 μg); (lane 2) kidney RNA (50 μg); (lane 3) lung RNA (50 μg). Exposure time, 48 hr. Note, the results of Figures 1 and 2 were obtained using different batches of RNA samples from the control untreated rats. We have purposedly overexposed this autoradiogram to detect the low level of kininogen expression in the kidney.

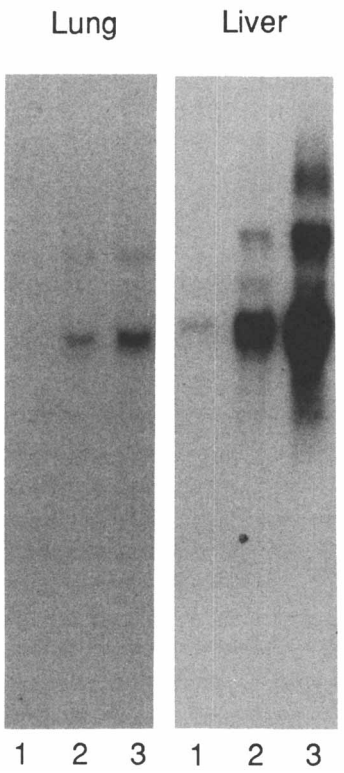

Figure 2. Northern blot analysis of kininogen mRNA in control and MCT-treated Sprague-Dawley rats. Kininogen mRNA levels were probed with a kininogen cDNA probe. (Left panel) Lung RNA (50 μg). (Right panel) Liver RNA (10 μg). (Lane 1) Control rat; (lane 2) 10 days after MCT injection; (lane 3) 20 days after MCT injection. Exposure time, 24 hr.

antiserum (22). Briefly, the nitrocellulose filter was blocked with BLOTTO (5% [w/v] dry milk in 0.01 *M* phosphate [pH 7.4], 0.14 *M* NaCl, 1 μM *para*-amidinophenylmethylsulfonylfluoride, 1 mg/liter thimerosal, 200 mg/liter NaN_3, and 0.01% antifoam A) for 1 hr at 30°C and then incubated with rabbit antirat T-kininogen antiserum (1:250 in BLOTTO). After a 3-hr incubation at 30°C with gentle shaking, the nitrocellulose membranes were washed three times with BLOTTO and then incubated with ^{125}I-labeled T-kininogen (250,000 cpm/ml) for 1.5 hr at 30°C. The nitrocellulose membranes were washed three times with BLOTTO and once with phosphate-buffered saline (0.01 *M* sodium phosphate and 0.14 *M* NaCl, pH 7.4), dried, and exposed to Kodak X-Omat film (Eastman Kodak, Rochester, NY).

RNA Extraction. Liver or lung was removed after perfusion with normal saline, as described above, and then minced and homogenized with a Polytron (1 min) in GIT buffer (4 *M* guanidine thiocyanate, 25 m*M* sodium acetate, and 0.1 *M* 2-mercaptoethanol). The homogenate was ultracentrifuged (174,000*g* at 20°C for 21 hr) over a cesium chloride discontinuous gradient (5.7 *M* CsCl and 25 m*M* sodium acetate). The resulting RNA pellet was dissolved in diethyl pyrocarbonate-treated water, and the RNA concentration was determined in a spectrophotometer by absorbance at 260 nm.

cDNA Probe Preparations. Cloning of a rat kininogen cDNA was described by Chao *et al.* (21). A double-stranded kininogen cDNA insert, about 1200 base pairs in length, was prepared from M13 mp18 DNA by restriction digestion (*Eco*RI/*Bam*HI), followed by agarose gel electrophoresis and electroelution. Rat α_1-antitrypsin and kallikrein-binding protein cDNAs were prepared as described previously (24, 25). The cDNA probes were labeled with ^{32}P (New England Nuclear Research Products, Boston, MA), using a nick-translation kit (Bethesda Research Laboratories, Gaithersburg, MD) according to the procedures recommended by the supplier. Unincorporated label was removed using a spin column (G-50, Gibco/Bethesda Research Laboratories, Grand Island, NY), and the specific activity of each probe was around $1–2 \times 10^8$ cpm/μg DNA.

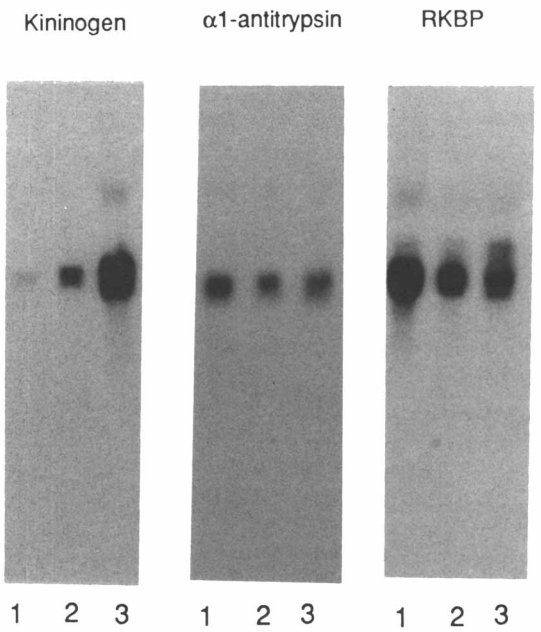

Figure 3. Northern blot analysis of liver mRNA for kininogen, α_1-antitrypsin, and rat kallikrein-binding protein (RKBP) in MCT-treated rats. (Left panel) Liver RNA (10 μg) was probed with a kininogen cDNA probe. (Middle panel) Liver RNA (10 μg) was probed with an α_1-antitrypsin cDNA probe. (Right panel) Liver RNA (10 μg) was probed with a RKBP cDNA probe. (Lanes 1) Control rat; (lanes 2) 10 days after MCT injection; (lanes 3) 20 days after MCT injection.

Northern Blot Analysis. Total RNA from liver or lung was denatured in a solution containing 1 × MOPS buffer (5 × MOPS buffer = 0.1 *M* MOPS [pH 7.0], 20 m*M* sodium acetate, and 0.5 m*M* EDTA), 50% formamide, and 2.2 *M* formaldehyde at 65°C for 15 min. Denatured RNA samples were resolved in a 1.5% agarose gel containing 2.2 *M* formaldehyde in 1 × MOPS buffer. The gel was run at 100 V with constant voltage for 2 hr. RNA was visualized by ethidium bromide staining and was transferred to an Immobilon-N membrane according to the protocols suggested by the manufacturer. The blot was baked at 80°C for 2 hr and prehybridized with a solution containing 6 × SSC (1 × SSC = 0.15 *M* NaCl and 0.015 *M* sodium acetate), 5 × Denhardt's solution (50 × Denhardt's solution = 1% Ficol, 1% polyvinylpyrrolidone, and 1% bovine serum albumin), 0.5% SDS, and 100 μg/ml herring sperm DNA at 60°C for 2 hr. Nick-translated cDNA probes (kininogen, α_1-antitrypsin, and kallikrein-binding protein) were added to the solution at a final concentration of 3×10^5 cpm/ml, and hybridization was carried out for 12 hr at 60°C. The blot was washed to a final stringency of 0.5 × SSC-0.1% SDS at 60°C, and autoradiography was carried out with an intensifying screen at −80°C. Autoradiographs (Kodak XAR-5 film) were obtained and quantified by densitometry.

Immunocytochemistry. Small pieces of the tissues to be used for biochemical analyses were taken for light microscopic immunocytochemistry and processed by standard procedures, summarized as follows. Tissues were fixed by immersion in calcium-acetate-buffered formalin, dehydrated in graded alcohols, and embedded in Paraplast. Five-micron sections were cut, and immunocytochemistry was performed using an indirect immunoperoxidase method, summarized as follows. Rehydrated tissues were incubated in primary antibody (rabbit antirat T-kininogen) at dilutions ranging from 1:50 to 1:100, either for 2 hr or overnight, followed by 0.5-hr incubation in peroxidase-conjugated goat anti-rabbit immunoglobulin (Cappel/Organon Chemical, Durham, NC). Color was developed using a 3,3′-diaminobenzidine incubation with H_2O_2 in 0.05 *M* Tris buffer, followed by a brief (1-sec) dip in 1% OsO_4. Sections were rinsed carefully in phosphate-buffered saline between each step, then dehydrated and mounted in Permount. Normal serum controls consisted of substituting either normal rabbit serum obtained commercially or a preimmune serum, for the primary antiserum at the same dilutions, i.e., 1:50 or 1:100 in the initial incubation step.

Statistical Analysis. Data are expressed as the mean ± standard error of the mean. Differences were assessed by analysis of variance. Differences were considered significant at a level of $P < 0.05$.

Results

Tissue-Specific Expression of the Kininogen Gene. Tissue-specific expression of the kininogen gene in the rat was analyzed by Northern blot analyses using a kininogen cDNA probe. LMW kininogen transcripts were identified as a 1.6-kilobase mRNA expressed in liver, kidney, and lung (Fig. 1). Liver displayed the highest level of kininogen mRNA (lane 1) and is the major site of kininogen synthesis. Kininogen mRNA in the kidney was barely detectable by the kininogen cDNA probe (lane 2), whereas lung expressed moderate levels of LMW kininogen mRNA (lane 3). Kininogen mRNAs were not detected in other tissues under these experimental conditions (data not shown).

MCT-Induced Kininogen Gene Expression in Lung and Liver. Northern blot hybridization showed that 10 and 20 days after a single injection of MCT, kininogen mRNA levels in lung increased 7.5- and 13.7-fold, respectively (Fig. 2, left panel). The autoradiograms were quantified by densitometry. Similarly, the LMW kininogen mRNA levels in liver after 10 and 20 days of MCT treatment increased 10.4- and 14.3-fold, respectively (Fig. 2, right panel). In contrast, when the same blot was stripped and reblotted with an α_1-antitrypsin (24) or rat kallikrein-binding protein (25) cDNA probe, MCT administration resulted in a slight decrease in both α_1-antitrypsin and kallikrein-binding protein mRNA levels in the liver (Fig. 3, middle and right panels). The results clearly indicate that MCT specifically induces kininogen gene expression in both

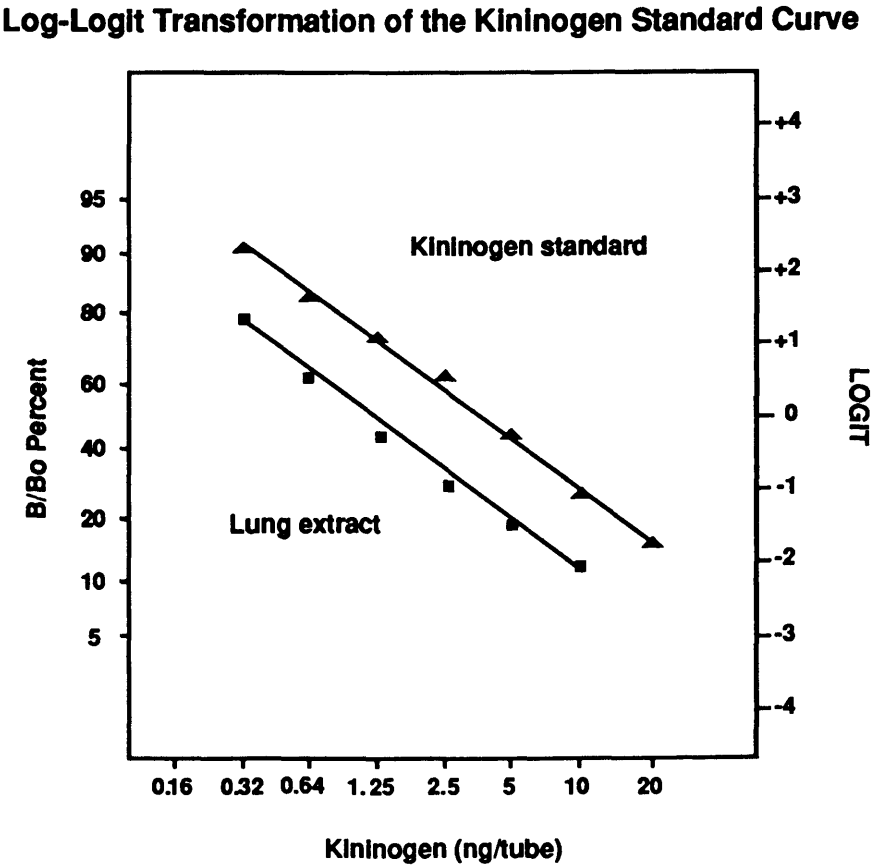

Figure 4. Log-logit transformation of a typical RIA standard curve of rat kininogen (▲), serial dilutions of normal rat lung extract (■). B/Bo is the amount of bound radioactivity in the presence divided by that in the absence of unlabeled kininogen, using rabbit antirat kininogen antiserum. The ratio is expressed as a percentage.

Table I. Effect of MCT on Kininogen Levels in Rat Serum, Lung, and Liver

Treatment	Days postinjection	Serum (μg/ml)	Immunoreactive kininogen (μg/mg of protein)	
			Lung	Liver
Vehicle	20	332 ± 19	0.8 ± 0.1	0.32 ± 0.10
MCT	10	989 ± 164[a]	6.2 ± 2.3[a]	0.65 ± 0.12[b]
MCT	20	2750 ± 941[a]	16.0 ± 3.9[b]	1.13 ± 0.17[b]

Kininogen levels were measured by a direct RIA. Values shown are the mean ± standard error (n = 6) in each group.
[a] $P < 0.05$ vs. control rats.
[b] $P < 0.01$ vs. control rats.

lung and liver, but has no significant effect on the expression of two other hepatocyte proteins, rat α_1-antitrypsin and kallikrein-binding protein.

MCT-Induced Kininogen Levels in Sera, Lung, and Liver. Using a direct RIA developed for rat T-kininogen (20) (which measures both LMW T- and K-kininogens), we detected immunoreactive kininogen in perfused lung extracts. A log-logit transformation of kininogen RIA, using a serial dilution of the lung tissue extracts, showed parallelism to the standard curve of purified T-kininogen in the direct RIA (Fig. 4). The results indicate immunological identity between pulmonary kininogen and rat LMW kininogen. Table I shows the effect of MCT administration on kininogen levels in serum, lung, and liver. Kininogen levels in serum increased 3- and 9-fold after a single injection of MCT for 10 and 20 days, respectively. Kininogen levels in lung increased 7- and 20-fold, respectively, after 10 and 20 days of MCT administration. Similarly, kininogen levels in liver increased 2- and 4-fold, respectively, after MCT treatment for 10 and 20 days.

Western Blot Analysis of Immunoreactive Kininogen in Lung. In Western blot analysis using a specific antigen overlay method, antibody against rat T-kini-

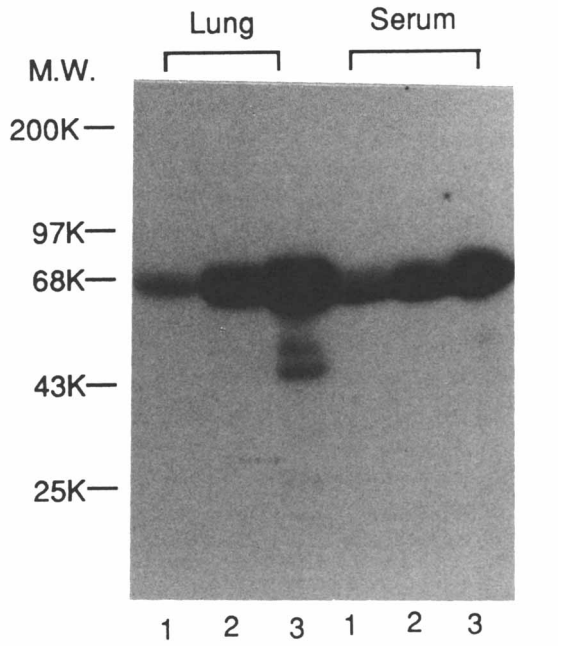

Figure 5. Western blot analysis of kininogen levels in rat lung and serum after MCT administration. Rat serum (1 μl) or lung extract (50 μg) was electrophoresed in a linear gradient (7.5–15%) polyacrylamide gel containing 0.1% SDS and transferred to nitrocellulose. The blot was incubated with kininogen polyclonal antibodies, followed by ^{125}I-labeled kininogen. (Lanes 1) Normal rat samples; (lanes 2) 10 days after MCT injection; (lanes 3) 20 days after MCT injection. M.W., Molecular weight.

nogen recognizes a 68-kilodalton kininogen in serum and lung extracts (Fig. 5, lanes 1), and the immunoreactive kininogen levels in both serum and lung were increased 10 days (Fig. 5, lanes 2) and 20 days (Fig. 5, lanes 3) after MCT treatment. The results are consistent with those determined by RIA (Table I).

Effect of MCT on Cellular Localization of Kininogen in Lung: In sections of lung from animals not injected with MCT, staining for kininogen was most obvious in endothelium of blood vessels and in cells within alveoli, presumably macrophages. The endothelium of both small and medium-sized veins stained especially intensely (Fig. 6a); the endothelium of medium-sized arteries was often stained, although not as intensely. Staining in capillary endothelium was seen only in a patchy distribution, but normal serum control sections exhibited virtually no staining (not shown).

In lung sections from MCT-injected animals, immunostaining for kininogen was much more intense and widely distributed than in sections from noninjected animals (Fig. 6b). Staining appeared to be present in virtually all blood vessels and even coated alveolar surfaces. In addition, staining for kininogen was seen in the connective tissue stroma surrounding bronchioles and blood vessels. Control sections (that is, sections incubated with normal rabbit serum in place of antikininogen antiserum) from MCT-treated animals exhibited staining in mast cells around bronchioles and arteries; such nonspecific mast cell staining is not uncommon, but it underscored an increased prevalence of mast cells in lungs of MCT-treated animals.

Discussion

This is the first study to demonstrate that rat LMW kininogen gene expression is induced in the rat lung, and that its pulmonary localization is abnormal 20 days after a single injection of MCT. Tissue-specific expression of kininogen in normal Sprague-Dawley rats was identified in the liver, lung, and kidney by Northern blot analysis. The induction of kininogen gene expression in the lung by MCT is time dependent, as shown by Northern blot analysis, RIA, and Western blot. Using a polyclonal T-kininogen antibody for immunocytochemistry, we also localized kininogen in the lung and found that its tissue distribution is altered after MCT treatment.

In Northern blot analysis using a 1200-base pair kininogen cDNA probe, we found that LMW kininogen gene expression in lung and liver is induced after MCT injection. Since rat LMW kininogens (K- and T-kininogens) share a high degree of sequence identity (26), and their cDNAs cross-react, we could not distinguish which species of kininogen mRNA (K- or T-kininogen) is induced by MCT using a kininogen cDNA probe. Similarly, the antibody to rat T-kininogen can recognize K-kininogen in the tissue extracts. Our previous studies showed that acute inflammation induced by the administration of endotoxin in rats exhibited a time-dependent increase in rat α_1-antitrypsin mRNA levels, while the same treatment reduced rat kallikrein-binding protein (27). The results indicated that the effect of MCT on kininogen gene expression in lung and liver is specific and is not ascribed to a general inflammatory phenomenon.

MCT administration has been shown to injure lung and kidney in addition to liver (28). Kininogens may participate in blood pressure homeostasis and inflammation as both precursors of potent vasoactive kinin peptides and cysteine proteinase inhibitors. The present finding that kininogen gene expression is induced in the lung after MCT-induced pulmonary vascular injury in a time-dependent manner suggests the possibility of a role for kininogen in this pulmonary hypertensive rat model.

The immunohistochemical results indicate that kininogen in normal lung is restricted to two sites: vascular endothelium and alveolar macrophages. The most intense staining was in the endothelium of small and medium-sized veins, as judged by two criteria: morphology and distribution. Veins had little or no smooth muscle in their walls, and they tended to be separated from bronchioles, whereas arteries had a muscular media and were found in the same stromal compartments as bronchioles. After MCT-induced pul-

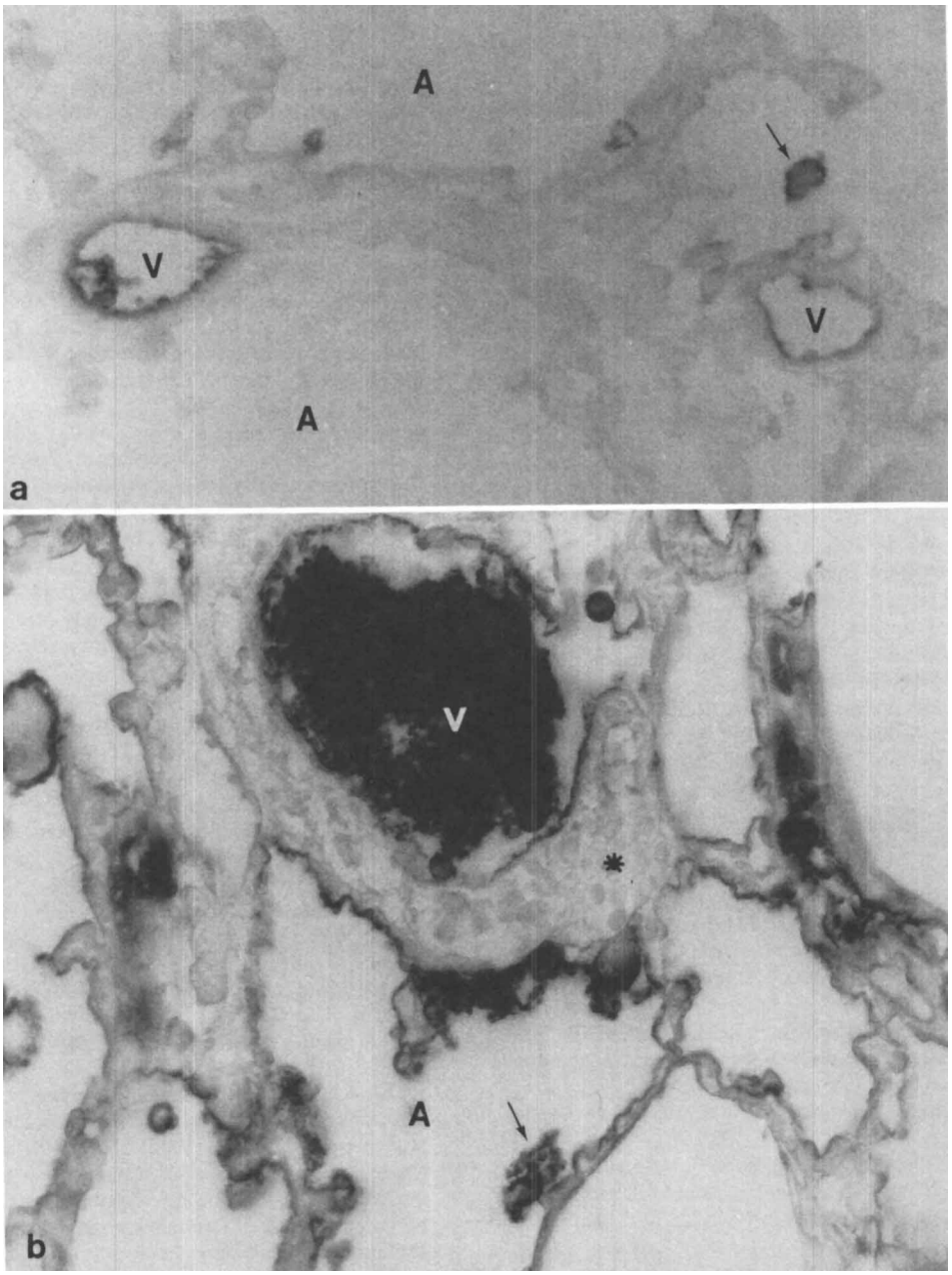

Figure 6. Lungs, stained for kininogen (rabbit anti-T-kininogen, 1:100 dilution); ×600 magnification. (a) Control rat, illustrating two immunoreactive blood vessels (V) in septa between alveoli (A). In addition to the staining in vascular endothelium, alveolar macrophages (arrow) are highly immunoreactive. (b) Rat injected with MCT 20 days previously. In addition to intense intravascular staining (V), alveolar surfaces were often covered with immunoreactive material, and alveolar macrophages (arrows) appear increased in number. In addition, inflammatory cells (*), including lymphocytes, macrophages, and mast cells have accumulated in thickened vascular walls.

monary damage, two changes in kininogen distribution were evident. First, an increased intensity of staining in vascular channels and pulmonary macrophages was obvious. Secondly, kininogen often covered the alveolar surface. This latter localization could result from either discharge of kininogen from alveolar macrophages or leakage of kininogen through damaged endothelial cells. The increase in stromal mast cells after MCT treatment could result in increased vascular permeability in these regions.

The finding that the kininogen gene is expressed in the lung and that its synthesis and localization in the lung are altered after MCT administration implicate kininogens in certain forms of lung injury, remodeling, and/or pulmonary hypertension. Kininogen, as a cysteine proteinase inhibitor, may participate in preventing the activation of such inflammatory cells as polymorphonuclear leukocytes and platelets, thus protecting endothelial cells from inflammatory insults. The anti-inflammatory action of kininogen can be eliminated if

the molecule undergoes proteolytic cleavage by proteinases, releasing peptides that include proinflammatory kinins. The role of kininogen and kinin in the lung injury and tissue remodeling remains to be elucidated.

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1. Sugita T, Hyers TM, Dauber IM, Wagner WW, McMurtry IF, Reeves JT. Lung vessel leak precedes right ventricular hypertrophy in monocrotaline-treated rats. J Appl Physiol **54**:371–374, 1983.
2. Stenmark KR, Morganroth ML, Remigo LK, Voelkel NF, Murphy RC, Henson PM, Mathias MM, Reeves JT. Alveolar inflammation and arachiconate metabolism in monocrotaline-induced pulmonary hypertension. Am J Physiol **248**:859–866, 1985.
3. Meyrick B, Gamble W, Reid L. Development of *Crotalaria* pulmonary hypertension: Hemodynamic and structural study. Am J Physiol **239**:H692-H702, 1980.
4. Rosenberg HC, Rabinovitch M. Endothelial injury and vascular reactivity in monocrotaline pulmonary hyperteinsion. Am J Physiol **255**:H1484–H1491, 1988.
5. Gillespie MN, Goldblum SE, Cohen DA, McClain CJ. Interleukin-1 bioactivity in the lungs of rats with monocrotaline-induced pulmonary hypertension. Proc Soc Exp Biol Med **187**:26–32, 1988.
6. Orlinska U, Olson JW, Gillespie MN. Polyamine content in pulmonary arteries from rats with monocrotaline-induced pulmonary hypertension. Res Commun Chem Pathol Pharmacol **62**:187–194, 1988.
7. Kay JM, Keane PM, Suyama KL, Gauthier D. Angiotensin converting enzyme activity and evolution of pulmonary vascular disease in rats with monocrotaline pulmonary hypertension. Thorax **37**:88–96, 1982.
8. Todorovich-Hunter L, Johnson DJ, Ranger P, Keeley FW, Rabinovitch M. Altered elastin and collagen synthesis associated with progressive pulmonary hypertension induced by monocrotaline: A biochemical and ultrastructural study. Lab Invest **58**:184–195, 1988.
9. Ito K, Hayashi K, Nakashima T, Murakami T. Alteration of fate of vasoactive autacoids in pulmonary circulation following monocrotaline-induced lung vascular injury in rats. Br J Pharmacol **94**:1167–1174, 1988.
10. Guzowski DE, Salgado ED. Changes in main pulmonary artery of rats with monocrotaline-induced pulmonary hypertension. Arch Pathol Lab Med **111**:741–745, 1987.
11. Hayashi Y, Lalich JJ. Renal and pulmonary alterations induced in rats by a single injection of monocrotaline. Proc Soc Exp Biol Med **124**:392–396, 1967.
12. Müller-Esterl W, Iwanaga S, Nakanishi S. Kininogens revisited. TIBS **11**:336–339, 1986.
13. Kato H, Nagasawa S, Iwanaga S. HMW and LMW kininogens. Methods Enzymol **80**:172–198, 1981.
14. Barlas A, Okamoto H, Greenbaum LM. T-kininogen–The major plasma kininogen in rat adjuvant arthritis. Biochem Biophys Res Commun **129**:280–286, 1985.
15. Johnson AR. Bradykinin, kallidin and kallikrein. In: Erdos EG, Ed. Effects of Kinins on Organ Systems. Pharmacol. New York: Springer-Verlag, Vol **25**(Suppl): pp357–399, 1979.
16. Ellis EF, Chao J, Heizer ML. Brain kininogen following traumatic brain injury: evidence for a secondary event. J Neurosurg **71**:437–442, 1989.
17. Xu J, Hsu CY, Junker H, Chao S, Hogan EL, Chao J. Kininogen and kinin in experimental spinal cord injury. J Neurochem **57**:974–980, 1991.
18. Chagas JR, Hirata IY, Juliano MA, Xiong W, Wang C, Chao J, Juliano L, Prado ES. Substrate specificities of tissue kallikrein and T-kininogenase: Their possible role in kininogen processing. Biochemistry **131**:4969–4974, 1992.
19. Chao J, Swain CC, Chao S, Xiong W, Chao L. Tissue distribution and kininogen gene expression after acute-phase inflammation. Biochim Biophys Acta **964**:329–339, 1988.
20. Chao S, Chao L, Chao J. Sex dimorphism and inflammatory regulation of T-kininogen and T-kininogenase. Biochim Biophys Acta **991**:477–483, 1989.
21. Chao S, Chao J, Chao L. Immunoscreening of cDNA expression libraries. Methods Nucleic Acids Res **307**:319, 1990.
22. Chen L, Chung P, Chao S, Chao L, Chao J. Differential regulation of kininogen gene expression by estrogen and progesterone *in vivo*. Biochim Biophys Acta **1131**:145–151, 1992.
23. Lowry OH, Rosebrough NJ, Farr AL, Randall JR. Protein measurement with the Folin phenol reagent. J Biol Chem **193**:265–275, 1951.
24. Chao S, Chai KX, Chao L, Chao J. Molecular cloning and primary structure of rat α1-antitrypsin. Biochemistry **29**:323–329, 1990.
25. Chao J, Chai KX, Chen LM, Xiong W, Woodley-Miller C, Wang L, Lu HS, Chao L. Tissue kallikrein-binding protein: Purification, characterization and distribution in normotensive and hypertensive rats. J Biol Chem **265**:16394–16401, 1990.
26. Furuto-Kato S, Matsumoto A, Kitamura N, Nakanishi S. Primary structures of the mRNAs encoding the rat precursors for bradykinin and T-kinin. J Biol Chem **260**:12054–12059, 1985.
27. Chao J, Chen LM, Chai KX, Chao L. Expression of kallikrein-binding protein and α1-antitrypsin genes in response to sex hormones, growth, inflammation and hypertension. In: Fritz H, Müller-Esterl W, Jochum M, Roscher A, Luppertz K, Eds. Recent Progress on Kinins: Biochemistry and Molecular Biology of the Kallikrein-Kinin System. Agents and Actions Supplements. Basel: Birkhauser, Vol **38(I)**: pp174–181, 1992.
28. Hayashi Y, Lalich JJ. Renal and pulmonary alterations induced in rats by a single injection of monocrotaline. Proc Soc Exp Biol Med **124**:392–396, 1967.