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Monocrotaline-induced pulmonary hypertension correlates with upregulation of connective tissue growth factor expression in the lung

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Abbreviations: CTGF, connective tissue growth factor; ET-1, endothelin-1; MCT, monocrotaline; PH, pulmonary hypertension; TGF-β1, transforming growth factor-β1; VSMC, vascular smooth muscle cell

Abstract

Pulmonary hypertension (PH) is characterized by structural and functional changes in the lung including proliferation of vascular smooth muscle cells (VSMCs) and excessive collagen synthesis. Although connective tissue growth factor (CTGF) is known to promote cell proliferation, migration, adhesion, and extracellular matrix production in various tissues, studies on the role of CTGF in pulmonary hypertension have been limited. Here, we examined CTGF expression in the lung tissues of male Sprague Dawley rats treated with monocrotaline (MCT, 60 μg/kg), a pneumotoxic agent known to induce PH in animals. Establishment of PH was verified by the significantly increased right ventricular systolic pressure and right ventricle/left ventricle weight ratio in the MCT-treated rats. Histological examination of the lung revealed profound muscular hypertrophy in the media of pulmonary artery and arterioles in MCT-treated group. Lung parenchyma, vein, and bronchiole did not appear to be affected. RT-PCR analysis of the lung tissue at 5 weeks indicated significantly increased expression of CTGF in the MCT-treated group. *In situ* hybridization studies also confirmed abundant CTGF mRNA expression in VSMCs of the arteries and arterioles, clustered pneumocytes, and infiltrated macrophages. Interestingly, CTGF mRNA was not detected in VSMCs of vein or bronchiole. In saline-injected control, basal expression of CTGF was seen in bronchial epithelial cells, alveolar lining cells, and endothelial cells. Taken together, our results suggest that CTGF upregulation in arterial VSMC of the lung might be important in the pathogenesis of pulmonary hypertension. Antagonizing the role of CTGF could thus be one of the potential approaches for the treatment of PH.

Keywords: connective tissue growth factor; fibrosis; hypertrophy; monocrotaline; pulmonary hypertension

Introduction

Pulmonary hypertension (PH) is a rare lung disorder in which the blood pressure in the pulmonary artery rises far above normal levels and may become life threatening (D'Alonzo *et al.,* 1991). It is characterized by structural and functional changes in pulmonary vasculature and proliferation of pulmonary artery smooth muscle cells as well as excess collagen synthesis in the lung (Botney *et al.,* 1993). Although pathogenesis of PH involves a complex and multifactorial process, current evidence suggests that endothelial dysfunction plays an integral role in the initiation and progress of PH (Veyssier-Belot and Cacoub, 1999; Budhiraja *et al.,* 2004).

Monocrotaline (MCT), a pyrrolizidine alkaloid from *Crotalaria spectabilis*, is activated metabolically in the liver to monocrotaline pyrrole which is then transported to the lungs and becomes pneumotoxic (Wilson *et al.,* 1992). It is thought to induce lung reactions such as interstitial edema, inflammation, hemorrhage and fibrosis (Kay and Heath, 1969). Since subcutaneous injection of MCT can cause PH, medial hypertrophy of the pulmonary arteries, and severe pres-

sure overload-induced right ventricular hypertrophy, MCT has been widely used to establish an animal model of PH (Meyrick *et al.,* 1980; Ghodsi and Will, 1981).

Connective tissue growth factor (CTGF) is a cysteine-rich, 38-kDa polypeptide that was originally isolated from human umbilical vein endothelial cells (Bradham *et al.,* 1991) and subsequently found in many cells including fibroblasts, chondrocytes, and smooth muscle cells (Kothapalli *et al.,* 1997; Nakanishi *et al.,* 1997; Hishikawa *et al.,* 1999b). CTGF is involved in many cellular processes underlying fibrosis such as cell proliferation, migration, adhesion, and the synthesis of extracellular matrix (ECM) (Brigstock, 1999). It induces expression of fibronectin and collagen type I, which are the molecules abnormally deposited in fibrotic lesions of major organs such as liver, kidney, lung, and skin (Igarashi *et al.,* 1996; Lasky *et al.,* 1998; Ito *et al.,* 1998; Paradis *et al.,* 1999). Accumulation of connective tissue in vessel wall is an important component of the alterations in PH and results from a complex interplay between synthesis and degradation of ECM (Vieillard-Baron *et al.,* 2000). Although there have been many studies on CTGF with regard to the development of fibrotic pathology (Grotendorst, 1997), its role in pulmonary hypertension has remained unexplored.

In the present study, we investigated CTGF expression in a rat model of MCT-induced PH. We first identified the regions of the lung that are affected by MCT treatment and then examined the level and distribution of CTGF expression by RT-PCR and *in situ* hybridization, respectively. Elevated expression of CTGF was observed in the hypertrophic smooth muscle cells of arteries and arterioles and in the cells that had infiltrated into the alveolar space of the lung, but not in the vein and bronchus. Our results suggest potential relevance of increased level of CTGF to the development of pulmonary arterial hypertrophy and PH in response to MCT.

Materials and Methods

Rat model of PH

MCT (300 mg Crotaline; Sigma, St. Louis, MI) was dissolved in 1.8 ml of 1 M HCl followed by addition of 3 to 4 ml of distilled water. This solution was adjusted to pH 7.4 using 1 M NaOH solution and filled up to 15 ml with distilled water (Hayashi *et al.,* 1967). Male Sprague-Dawley rats (6-week-old) received a single subcutaneous injection of MCT solution (60 mg/kg) or saline solution. Rats were housed with a 12/12 - light/dark cycle and given water and standard rat chow *ad libitum*. At 2 or 5 weeks after injection, the rats were sacrificed and the organs harvested for the following analyses.

Measurement of hemodynamic parameters and assessment of right ventricular hypertrophy

Rats were anesthetized using an intramuscular injection of ketamine (70 mg/kg; Ketalar 50 mg/ml; Yuhan Co., Korea) and xylazine (10 mg/kg; Rompun 23.32 mg/ml; Bayer, Korea). After exposing the heart, a 21-gauge needle was inserted into the right ventricle (RV). Pulmonary arterial pressures were measured immediately after insertion of the needle using the compact configurable monitor (78354C, HP, Palo Alto, CA). After the pressure measurement, the heart was excised and weighed. The weight ratio of RV free wall to septum plus left ventricular (LV) free wall was also measured.

Histology

The lung was prepared for histopathologic examination as follows. For paraffin section, the lung was isolated and fixed in formalin for 24 h at room temperature, dehydrated in ethanol, cleared in xylene, and embedded in paraffin block. Sections were cut in 6 μm thickness and subjected to Massons's trichrome staining for the presence of collagen, which is a typical indicator of fibrosis.

In situ hybridization

Non-radioactive RNA probes were generated by *in vitro* transcription from the plasmid containing the 5' portion of the CTGF cDNA using SP6 or T7 RNA polymerase (Promega, Madison, WI). The tissue

Table 1. Effects of MCT-treatment on hemodynamic and physiologic profiles of rat.

	2 week		5 week	
	Saline	MCT	Saline	MCT
Body weight (BW), g	267 ± 5	254 ± 8	331 ± 11	174 ± 30*
RV systolic pressure, mmHg	24 ± 1	42 ± 2*	32 ± 2	78 ± 26*
RV/BW, g/kg body wt	0.67 ± 0.03	0.74 ± 0.04	0.56 ± 0.02	1.41 ± 0.16*
LV/BW, g/kg body wt	2.48 ± 0.04	2.33 ± 0.05	2.21 ± 0.04	2.53 ± 0.18
RV/LV, g/g	0.27 ± 0.01	0.32 ± 0.02	0.26 ± 0.01	0.57 ± 0.11*

Abbreviations: RV, right ventricular weight; LV, left ventricular weight. Data are mean ± SEM. *: $P < 0.05$ vs. saline group.

sections were dewaxed in xylene and fixed for 10 min in PBS containing 4% paraformaldehyde. The sections were then treated with proteinase K (20 μg/ml) for 15 min to permeabilize the tissue and postfixed for 10 min with 4% paraformaldehyde solution. The sections were acetylated for 10 min with 0.1 M triethanol amine and acetic anhydride, and pre-hybridized for 1 h at 68°C. Hybridization mixture (5×Denhardt's solution containing 50% formamide, 2×SSC, 5% Dextran sulfate, 150 μg tRNA and 150 μg/ ml denatured salmon sperm DNA) was then poured onto the section and incubated overnight at 68°C in a moisture chamber with a digoxigenin-labeled CTGF- specific riboprobe. The next day, sections were washed at 50°C with 4×SSC solution for 30 min and then with 2×SSC solution containing 50% formamide for 30 min. For immunohistochemical detection of digoxigenin, the

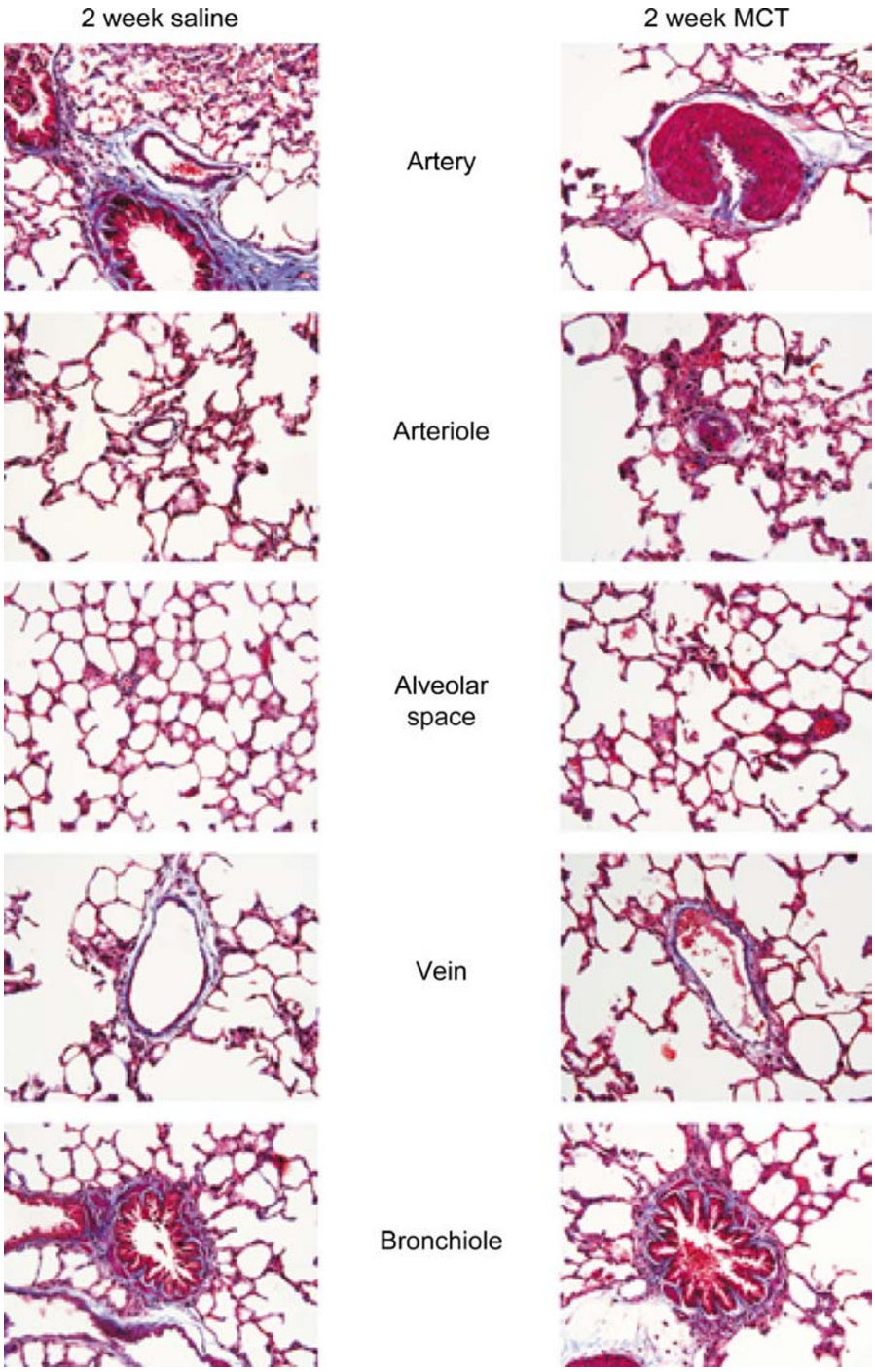

Figure 1. Photomicrograph of the lung sections stained with Masson's trichrome. Two weeks after MCT injection, muscular hypertrophy in the media of pulmonary artery and arteriole is evident. However, in the lung parenchyma, pulmonary vein, and bronchiole, no remarkable differences are seen between the saline- and MCT-injected groups. Blue staining indicates collagen deposition. Magnification: ×400.

sections were initially incubated in buffer A (100 mM Tris-HCl, pH 7.5 containing 150 mM NaCl) and then blocked at room temperature for 1 h in normal sheep serum. The sections were then incubated at 4°C overnight with alkaline phosphatase- conjugated sheep polyclonal anti-digoxigenin antibody (Roche, Mannheim, Germany) diluted to 1:1000 in buffer A containing 1% normal sheep serum. The following day, the sections were subjected to three cycles of 10 min washing with buffer A and buffer B (100 mM Tris-HCl, pH 9.5 containing 100 mM NaCl and 50 mM $MgCl_2$). Alkaline phosphatase detection was carried out in the presence of nitroblue tetrazolium, BCIP, and levamisol in buffer B at room temperature in the dark. The sections were then dehydrated and covered with glass coverslips.

RT-PCR

Total RNA was extracted from the lung using Qiagen RNeasy Mini Kit (Qiagen, Valencia, CA). After pre-heating 1 μg of RNA at 65°C for 10 min, first-strand

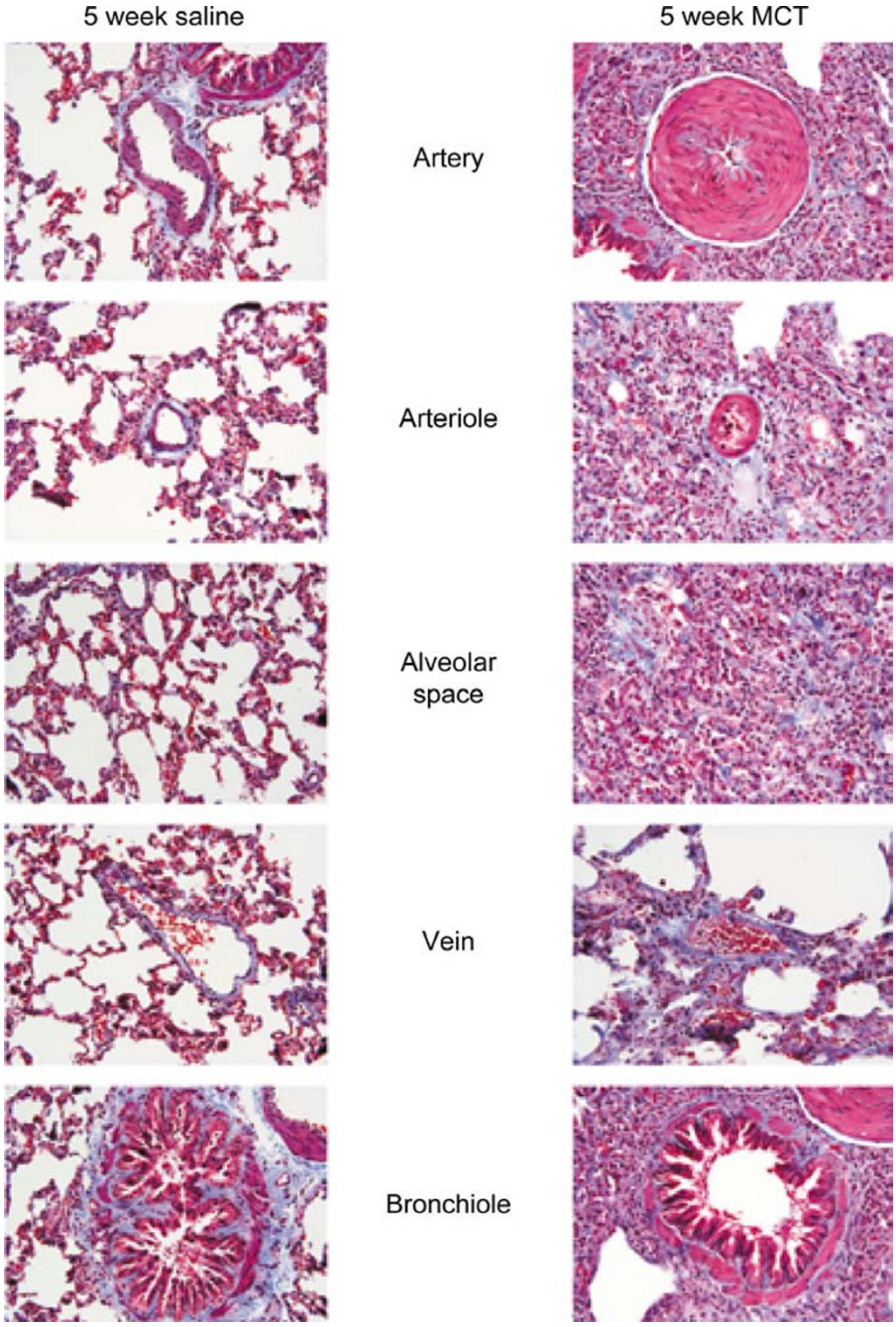

Figure 2. Photomicrograph of the lung sections stained with Masson's trichrome 5 weeks after MCT or saline injection. In addition to medial hypertrophy of pulmonary artery and arteriole, there are thickening of the alveolar septa with fibrosis, and alveolar macrophage infiltration in the lung treated with MCT. Bronchiole and vein do not appear to be affected by MCT treatment. Magnification: ×400.

cDNA synthesis was carried out at 42°C for 1 h using a random 6-mer primer and AMV reverse transcriptase (Promega) followed by denaturation at 95°C for 10 min. PCR amplification was performed using the CTGF-specific primers (5'-CCTGGTCCAGACCACAG-AGT-3' and 5'-CCAAGCTTCATGCCATGTCT-3') and transforming growth factor (TGF)-β1 primers (5'-GAA-CCAAGGAGACGGAATAC-3' and 5'-GACAGAAGTTG-GCATGGTAG-3').

Statistics

The data are expressed as means ± SEM. Differences between experimental groups were evaluated for statistical significance using Student's *t*-test and one-way ANOVA (Newman-Keuls multiple comparison test). A *P* value less than 0.05 was considered statistically significant.

Results

Rat model of pulmonary hypertension

A single subcutaneous injection of MCT resulted in a significant increase in RV systolic pressure after 5 weeks (78 ± 26 mm Hg) compared with the saline-injected control rats (32 ± 2 mm Hg) (Table 1). This was paralleled by right ventricular hypertrophy with the RV/LV weight ratio increase at 5 week (MCT groups: 0.57 ± 0.11; saline groups: 0.26 ± 0.01, respectively). Moreover, the MCT-injected rats (174 ± 30 g) had significantly lower body weight (BW) than the saline-injected controls (331 ± 11 g) after 5 weeks. The same tendency was noted at 2 week. The RV systolic pressure of the MCT group was significantly increased (42 ± 2 mm Hg) compared with that of the control (24 ± 1 mm Hg). However, differences in RV/LV ratio and BW between the two groups were not statistically significant at 2 week. These hemodynamic parameters indicated that a rat model of pulmonary hypertension was successfully made.

Hypertrophy of muscular pulmonary artery and arteriole and interstitial fibrosis of the lung following MCT treatment

Histological examination of the lung at 2 weeks after MCT treatment revealed profound muscular hypertrophy in the media of muscular pulmonary arteries and arterioles (Figure 1). In contrast, lung parenchyma, vein, and bronchiole did not appear to be affected. At 5 week, the MCT-treated lung displayed interstitial thickening and prominent medial hypertrophy of muscular pulmonary artery and arterioles (Figure 2). Fibrosis was demonstrated in thickened alveolar septa by Masson's trichrome stain. Intra-alveolar hemorrhage and macrophages were also found. These observations are in accordance with the previous report that showed the collagen deposition in the MCT-treated animals (Mansoor *et al.,* 1995).

CTGF upregulation in the lung of MCT-treated rat

CTGF has been implicated in the fibrosis of various tissues as described earlier. A possible effect of MCT treatment on the expression of CTGF mRNA was examined by RT-PCR analysis of the lung tissue. The result showed that higher level of CTGF mRNA was detected in the MCT-treated group at 2 and 5 week (Figure 3). In contrast, there was a little expression of CTGF mRNA in the saline-injected control group with negligible time-course changes. Interestingly, the level of TGF-β1 was significantly increased at 4 week, which appeared to precede the upregulation of CTGF at 5 week (Figure 3). An earlier report of CTGF expression induced by TGF-β1 (Hishikawa *et al.,* 1999a; Chen *et al.,* 2000) suggests that upregulation of TGF-β1 by MCT treatment may be responsible for the induction of CTGF expression.

Next, by *in situ* hybridization analysis of tissue sections, CTGF expressing cells in the lung were identified. As shown in Figure 4, CTGF mRNA was basally expressed in the endothelial cells of pulmonary artery and arteriole. Basal expression of CTGF was also observed in bronchial epithelium and alveolar lining cells. However, following MCT treatment, abundant expression of CTGF was observed in hypertrophic VSMCs of pulmonary artery and arteriole and in the clustered pneumocytes and macrophages that had infiltrated into alveolar space. Positive staining was also observed in alveolar septum. Interestingly, CTGF mRNA was not detected in VSMCs of vein or bronchiole.

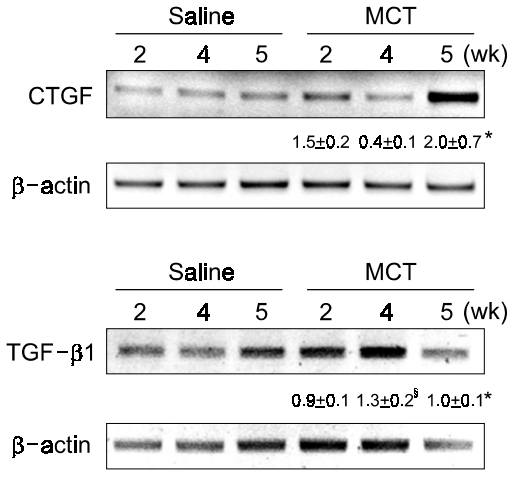

Figure 3. RT-PCR analysis of the lung tissue after saline or MCT treatment. Total RNA (1 μg) was isolated from each sample, reverse-transcribed, and then aliquot of the cDNA amplified by PCR using CTGF- or TGF-β1-specific primers. A second aliquot of the RT reaction was amplified using primers specific for β-actin as an internal control. One of the representative results is shown and the number indicates fold-increase over the saline control (mean ± SEM, $n = 6$). *$P < 0.05$ vs. 4 wk, §$P < 0.05$ vs. 2 wk

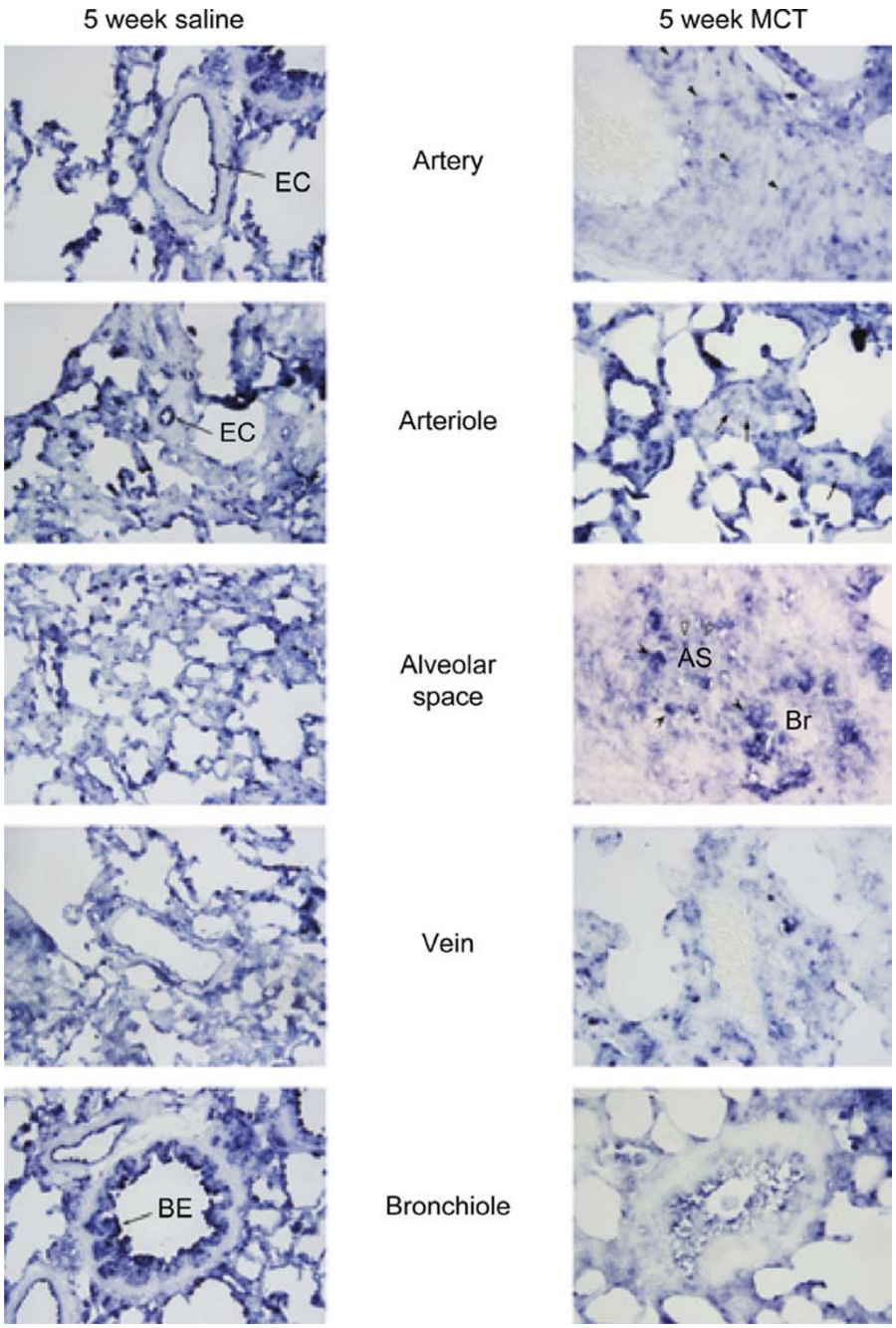

Figure 4. *In situ* hybridization of the lung tissue with CTGF mRNA-specific riboprobe. Five weeks after MCT treatment, abundant expression of CTGF mRNA is seen in smooth muscle cells of the remodeling pulmonary artery and arteriole (marked by black arrowhead and black arrow, respectively) and in infiltrated macrophages (marked by white arrowhead) within alveolar space (AS). Positive staining at alveolar septum is also marked by wedge. Endothelial dysfunction in the MCT-treated pulmonary artery is seen in support of previous study (Jones *et al.,* 1996). Basal expression of CTGF is noted in the endothelial cells (EC) of pulmonary artery and arteriole, bronchial epithelium (BE), and alveolar lining cells as shown in saline-injected controls. CTGF mRNA is not detected in vascular smooth muscle cells of the vein or bronchiole. Magnification: ×400.

Discussion

In the present study, we demonstrated that CTGF was highly upregulated in the lung of rat treated with MCT. Although it is known that CTGF is one of the key molecules in the progression of tissue fibrosis, this study is the first to suggest direct correlation between CTGF and MCT-induced PH *in vivo*. *In situ* hybridization study of the lung section showed that CTGF expression was mainly localized in the hypertrophic smooth muscle cells of pulmonary arteries and arterioles as well as in alveolar macrophages and pneumocytes. In support of this result, obliteration of artery, arteriole, and significant fibrosis of alveolar septa were observed in MCT-treated rat lung. Recent report that adenovirus-mediated overexpression of CTGF caused increased deposition of collagen *in vivo* (Bonniaud *et al.,* 2003) is consistent with our data and suggests that CTGF might play an important role in the development of PH.

Although a number of different mechanisms would be involved in CTGF induction, TGF-β1 is one of the potential mediators of CTGF upregulation in PH. Consistent with our finding (Figure 3), TGF-β1 ex-

pression was shown to be elevated in the lung during development of PH (Arcot *et al.,* 1993; Tanaka *et al.,* 1996). In addition, TGF-β1 can induce the synthesis of CTGF both transcriptionally and translationally (Igarashi *et al.,* 1993; Grotendorst *et al.,* 1996), thus promoting the production and accumulation of ECM components in certain pathological states including the fibrosis of the liver, kidney, and lung (Border and Noble, 1994). Another possible mediator of CTGF upregulation is endothelin (ET)-1, a 21-amino-acid peptide with potent vasoconstrictor activity and platelet-aggregating properties (Yanagisawa *et al.,* 1988). ET-1 was shown to induce expression of matrix and matrix-associated genes including CTGF in various cell types (Chaqour *et al.,* 2002; Shi-Wen *et al.,* 2004). The importance of ET-1 is also suggested by the previous reports which showed that ET-1 is elevated in patients with PH (Giaid *et al.,* 1993) as well as in animal models of PH (Mansoor *et al.,* 1995; Frasch *et al.,* 1999). Therefore, it is very likely that TGF-β1, together with ET-1, plays an active role in the upregulation of CTGF in MCT-induced PH.

MCT-induced PH is also associated with cardiac remodeling including RV hypertrophy and interstitial fibrosis as evidenced by our data and other studies (Ghodsi and Will, 1981; Honda *et al.,* 1992). Previously, it was reported that expression of rennin-angiotensin system, TGF-β1, and ET-1 was enhanced in RV hypertrophy of MCT-induced PH rats (Park *et al.,* 2001). In a more recent publication, Ahmed *et al.* (2004) demonstrated that CTGF acts as a myocardial effector of Angiotensin II-induced myocardial remodeling in ischemic heart failure *via* AT_1 receptors in cardiac fibroblasts. Since Angiotensin-II is also known to stimulate synthesis of TGF-β1 and ET-1 (Ito *et al.,* 1993; Weber, 1997), these evidences altogether suggest that CTGF might be a more downstream target contributing to cardiac remodeling after PH. However, further investigation is needed to elaborate on this point.

There are two contradictory views regarding the function of CTGF in regulating the growth of VSMC. One report suggested that CTGF acts as an inhibitor of human aortic smooth muscle cell growth at least in part by inducing apoptosis (Hishikawa *et al.,* 1999a; b). The other report, on the contrary, suggested that CTGF is a growth factor for VSMC and that it may play a similar role in promoting proliferation and migration of VSMC and production of extracellular matrix such as collagen type I and fibronectin (Fan *et al.,* 2000). The results of present study support the latter hypothesis because CTGF was specifically expressed in VSMC of remodeling arteries and arterioles with medial hypertrophy. However, since CTGF may not be the sole factor contributing to hypertrophy of VSMC and lung fibrosis, more studies are required to resolve this controversy.

PH has been historically chronic and incurable with a poor survival rate. Recently, however, many approaches aimed at restoring the balance between various vasoactive mediators have shown beneficial effects (Budhiraja *et al.,* 2004). Indeed, in patients with PH, an altered production of various endothelial vasoactive mediators, such as nitric oxide, prostacycline, ET-1, serotonin, and thromboxane, has been increasingly recognized. As an extension of these approaches, a strategy that is based on antagonizing the role of CTGF would represent novel therapeutic modality for PH.

In conclusion, we present an evidence that CTGF expression is upregulated in the lung of MCT-induced pulmonary hypertensive rat and that it is localized in the remodeling pulmonary arteries and arterioles and in infiltrated inflammatory cells. Our results suggest that CTGF plays an important role in the development of lung pathogenesis caused by PH.

Acknowledgment

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References

Ahmed MS, Oie E, Vinge LE, Yndestad A, Oystein Andersen G, Andersson Y, Attramadal T, Attramadal H. Connective tissue growth factor - a novel mediator of angiotensin II-stimulated cardiac fibroblast activation in heart failure in rats. J Mol Cell Cardiol 2004;36:393-404

Arcot SS, Lipke DW, Gillespie MN, Olson JW. Alterations of growth factor transcripts in rat lungs during development of monocrotaline-induced pulmonary hypertension. Biochem Pharmacol 1993;46:1086-91

Bonniaud P, Margetts PJ, Kolb M, Haberberger T, Kelly M, Robertson J, Gauldie J. Adenoviral gene transfer of connective tissue growth factor in the lung induces transient fibrosis. Am J Respir Crit Care Med 2003;168:770-8

Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. N Engl J Med 1994;331:1286-92

Botney MD, Liptay MJ, Kaiser LR, Cooper JD, Parks WC, Mecham RP. Active collagen synthesis by pulmonary arteries in human primary pulmonary hypertension. Am J Pathol 1993;143:121-9

Bradham DM, Igarashi A, Potter RL, Grotendorst GR. Connective tissue growth factor: A cysteine-rich mitogen secreted by human vascular endothelial cells is related to the SRC-induced immediate early gene product CEF-10. J Cell Biol 1991;114:1285-94

Brigstock DR. The connective tissue growth factor/cysteine-rich 61/nephroblastoma overexpressed (CCN) family. Endocr Rev 1999;20:189-206

Budhiraja R, Tuder RM, Hassoun PM. Endothelial dysfunction in pulmonary hypertension. Circulation 2004;109:159-65

Chaqour B, Whitbeck C, Han JS, Macarak E, Horan P, Chichester P, Levin R. Cyr61 and CTGF are molecular markers of bladder wall remodeling after outlet obstruction. Am J Physiol - Endocrinol Metab 2002;283:E765-E774

Chen MM, Lam A, Abraham JA, Schreiner GF, Joly AH. CTGF expression is induced by TGF-β in cardiac fibroblasts and cardiac myocytes: a potential role in heart fibrosis. J Mol Cell Cardiol 2000;32:1805-19

D'Alonzo GE, Barst RJ, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, Fishman AP, Goldring RM, Groves BM, Kernis JT, *et al*. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. Ann Intern Med 1991;115:343-9

Fan WH, Pech M, Karnovsky MJ. Connective tissue growth factor (CTGF) stimulates vascular smooth muscle cell growth and migration *in vitro*. Eur J Cell Biol 2000;79:915-23

Frasch HF, Marshall C, Marshall BE. Endothelin-1 is elevated in monocrotaline pulmonary hypertension. Am J Physiol 1999;276:L304-L310

Ghodsi F, Will JA. Changes in pulmonary structure and function induced by monocrotaline intoxication. Am J Physiol 1981;240:H149-H155

Giaid A, Yanagisawa M, Langleben D, Michel RP, Levy R, Shennib H, Kimura S, Masaki T, Duguid WP, Stewart DJ. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. N Engl J Med 1993;328:1732-9

Grotendorst GR, Okochi H, Hayashi N. A novel transforming growth factor beta response element controls the expression of the connective tissue growth factor gene. Cell Growth Differ 1996;7:469-80

Hayashi Y, Hussa JF, Lalich JJ. Cor pulmonale in rats. Lab Invest 1967;16:875-81

Hishikawa K, Nakaki T, Fujii T. Transforming growth factor-beta (1) induces apoptosis via connective tissue growth factor in human aortic smooth muscle cells. Eur J Pharmacol 1999a;385:287-90

Hishikawa K, Oemar BS, Tanner FC, Nakaki T, Fujii T, Luscher TF. Overexpression of connective tissue growth factor gene induces apoptosis in human aortic smooth muscle cells. Circulation 1999b;100:2108-12

Honda M, Yamada S, Goto Y, Ishikawa S, Yoshikane H, Ishinaga Y, Kuzuo H, Morioka S, Moriyama K. Biochemical and structural remodeling of collagen in the right ventricular hypertrophy induced by monocrotaline. Jpn Circ J 1992;56: 392-403

Igarashi A, Okochi H, Bradham DM, Grotendorst GR. Regulation of connective tissue growth factor gene expression in human skin fibroblasts and during wound repair. Mol Biol Cell 1993;4:637-45

Igarashi A, Nashiro K, Kikuchi K, Sato S, Ihn H, Fujimoto M, Grotendorst GR, Takehara K. Connective tissue growth factor gene expression in tissue sections from localized scleroderma, keloid, and other fibrotic skin disorders. J Invest Dermatol 1996;106:729-33

Ito H, Hirata Y, Adachi S, Tanaka M, Tsujino M, Koike A, Nogami A, Murumo F, Hiroe M. Endothelin-1 is an autocrine/paracrine factor in the mechanism of angiotensin II-induced hypertrophy in cultured rat cardiomyocytes. J Clin Invest 1993;92:398-403

Ito Y, Aten J, Bende RJ, Oemar BS, Rabelink TJ, Weening JJ, Goldschmeding R. Expression of connective tissue growth factor in human renal fibrosis. Kidney Int 1998;53: 853-61

Jones PL, Rabinovitch M. Tenascin-C is induced with progressive pulmonary vascular disease in rats and is functionally related to increased smooth muscle cell proliferation. Circ Res 1996;79:1131-42

Kay JM, Heath D. *Crotalaria spectabilis:* The Pulmonary Hypertension Plant. 1969, Thomas, Springfield, IL

Kothapalli D, Frazier KS, Welply A, Segarini PR, Grotendorst GR. Transforming growth factor beta induces anchorage-independent growth of NRK fibroblasts via a connective tissue growth factor-dependent signaling pathway. Cell Growth Differ 1997;8:61-8

Lasky JA, Ortiz LA, Tonthat B, Hoyle GW, Corti M, Athas G, Lungarella G, Brody A, Friedman M. Connective tissue growth factor mRNA expression is upregulated in bleomycin-induced lung fibrosis. Am J Physiol - Lung Cell Mol Physiol 1998;275:L365-L371

Mansoor AM, Honda M, Saida K, Ishinaga Y, Kuramochi T, Maeda A, Takabatake T, Mitsui Y. Endothelin induced collagen remodeling in experimental pulmonary hypertension. Biochem Biophys Res Commun 1995;215:981-6

Meyrick B, Gamble W, Reid L. Development of Crotalaria pulmonary hypertension: hemodynamic and structural study. Am J Physiol 1980;239:H692-H702

Nakanishi T, Kimura Y, Tamura T, Ichikawa H, Yamaai Y, Sugimoto T, Takigawa M. Cloning of a mRNA preferentially expressed in chondrocytes by differential display-PCR from a human chondrocytic cell line that is identical with connective tissue growth factor (CTGF) mRNA. Biochem Biophys Res Commun 1997;234:206-10

Paradis V, Dargere D, Vidaud M, De Gouville AC, Huet S, Martinez V, Gauthier JM, Ba N, Sobesky R, Ratziu V, Bedossa P. Expression of connective tissue growth factor in experimental rat and human liver fibrosis. Hepatology 1999; 30:968-76

Park HK, Park SJ, Kim CS, Paek YW, Lee JU, Lee WJ. Enhanced gene expression of renin-angiotensin system, TGF-beta1, endothelin-1 and nitric oxide synthase in right-ventricular hypertrophy. Pharmacol Res 2001;43:265-73

Tanaka Y, Bernstein ML, Mecham RP, Patterson GA, Cooper JD, Botney MD. Site-specific responses to monocrotaline-induced vascular injury: evidence for two distinct mechanisms of remodeling. Am J Respir Cell Mol Biol 1996;15: 390-7

Veyssier-Belot C, Cacoub P. Role of endothelial and smooth muscle cells in the physiopathology and treatment management of pulmonary hypertension. Cardiovasc Res 1999;44: 274-82

Vieillard-Baron A, Frisdal E, Eddahibi S, Deprez I, Baker AH, Newby AC, Berger P, Levame M, Raffestin B, Adnot S, d'Ortho MP. Inhibition of matrix metalloproteinases by lung TIMP-1 gene transfer or doxycycline aggravates pulmonary hypertension in rats. Circ Res 2000;87:418-25

Weber KT. Fibrosis, a common pathway to organ failure: angiotensin II and tissue repair. Semin Nephrol 1997;17:

467-91

Wilson DW, Segall HJ, Pan LC, Lame MW, Estep JE, Morin D. Mechanisms and pathology of monocrotaline pulmonary toxicity. Crit Rev Toxicol 1992;22:307-25

Xu SW, Howat SL, Renzoni EA, Holmes A, Pearson JD, Dashwood MR, Bou-Gharios G, Denton CP, DuBois RM, Black CM, Leask A, Abraham DJ. Endothelin-1 induces expression of matrix-associated genes in lung fibroblasts through MEK/ERK. J Biol Chem 2004;279:23098-103

Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411-5