PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **180,** 112–120 (1985)

Monocrotaline-Induced Pulmonary Fibrosis in Rats: Amelioration by Captopril and Penicillamine (42151)

AGOSTINO MOLTENI,* WILLIAM F. WARD,† CHUNG-HSIN TS'AO,* NORMAN H. SOLLIDAY,‡§ AND MOYRA DUNNE§

*Departments of *Pathology, †Radiology, and ‡Medicine, Northwestern University Medical School, Chicago, Illinois 60611, and §Christ Hospital, Oak Lawn, Illinois 60453*

Abstract. The purpose of this study was to determine whether Captopril (an angiotensin converting enzyme inhibitor) or D-penicillamine (an inhibitor of collagen crosslinking) can ameliorate pulmonary fibrosis induced by the plant alkaloid monocrotaline. Rats were randomly assigned to one of six treatment groups: (1) control; (2) Captopril, 60 mg/kg/day, p.o.; (3) D-penicillamine, 30 mg/kg/day, p.o.; (4) monocrotaline, 2.4 mg/kg/day, p.o.; (5) monocrotaline plus Captopril, as above; (6) monocrotaline plus penicillamine, as above; and were killed after 6 weeks of continuous drug administration. Monocrotaline-treated rats exhibited several anatomic correlates of pulmonary hypertension, including cardiomegaly, right heart enlargement, and muscularization of the pulmonary arteries and arterioles. These monocrotaline reactions were accompanied by decreased lung activities of angiotensin converting enzyme (ACE) and plasminogen activator (PLA), indicative of endothelial dysfunction; and by increased lung hydroxyproline concentration, indicative of interstitial fibrosis. The presence of interstitial fibrosis was confirmed by electron microscopy. When given concomitantly with monocrotaline, both Captopril and penicillamine partially prevented the cardiomegaly, right heart enlargement, and vascular muscularization. Both agents also diminished the decreased lung PLA activity and increased hydroxyproline concentration observed in monocrotaline-treated animals. Neither modifying agent influenced the monocrotaline-induced decrease in lung ACE activity. Compared with control rats, the rats receiving Captopril alone exhibited decreased heart weight and increased serum ACE activity, and animals receiving penicillamine alone did not differ significantly from control animals for any of the endpoints studied. These data demonstrate that Captopril and penicillamine ameliorate monocrotaline-induced pulmonary fibrosis in rats. Penicillamine, known to inhibit radiation-induced lung injury, thus is shown to be effective in a second model of pulmonary fibrosis. Perhaps more importantly, the hydroxyproline data demonstrate that the ACE inhibitor Captropril exhibits antifibrotic activity in monocrotaline-treated rat lung.

Pulmonary hypertension induced by the plant alkaloid monocrotaline is both a veterinary and a human health hazard (1). Ultrastructural studies of monocrotaline-treated lung emphasize inflammatory and hemorrhagic reactions, although interstitial fibrosis also has been observed (2–7). Monocrotaline injury in the lung is accompanied by pulmonary endothelial dysfunction, including decreased angiotensin converting enzyme (ACE) activity (7–9), decreased 5-hydroxytryptamine clearance (10, 11), decreased plasminogen activator (PLA) activity (7), and increased prostacyclin (PGI_2) production (7). The pathogenesis of monocrotaline-induced pulmonary hypertension is not clear, although inappropriate ACE activity may play a role. Supporting this hypothesis is evidence that inhibitors of ACE activity partially prevent pulmonary hypertension induced by both monocrotaline (12, 13) and chronic hypoxia (14, 15). We noted, based solely on an evaluation of electron micrographs, that ACE inhibitors also reduce collagen accumulation in monocrotaline-treated rat lung (12, 13). To our knowledge, ACE inhibitors have not been reported to exhibit antifibrotic activity. Therefore the present study was conducted in order to examine this possibility in monocrotaline-treated lung, using a more quantitative index of fibrosis, i.e., hydroxyproline content, as well as two indices of endothelial function, ACE and PLA activities. The ACE inhibitor Captopril (SQ14225) was tested with a regimen known to ameliorate monocrotaline-induced pulmonary vascular injury (12,

0037-9727/85 $1.50

13), and was compared with D-penicillamine, an inhibitor of collagen maturation (16). Penicillamine has been shown to inhibit *radiation*-induced pulmonary fibrosis in rats (17). In the present study, animals consumed monocrotaline (2.4 mg/kg/day) continuously for 6 weeks, a treatment time known to produce significant pulmonary injury (7).

Materials and Methods. Male Sprague–Dawley rats (Harlan Industries, Madison, Wis.) weighing 200–225 g were housed at 23 ± 1°C, and were randomly assigned to one of six treatment groups: (1) control, received tap water and powdered Purina rat chow (Ralston–Purina Co., St. Louis, Mo.) *ad libitum;* (2) Captopril (60 mg/kg/day), received water containing 500 mg Captopril (SQ14225, E. R. Squibb and Sons, Princeton, N.J.) per liter and powdered rat chow; (3) penicillamine (30 mg/kg/day), received tap water and powdered rat chow containing 0.05% (w/w) D-penicillamine (free base, Sigma Chemical Co., St. Louis, Mo.); (4) monocrotaline (2.4 mg/kg/day), received water containing 20 mg monocrotaline (Aldrich Chemical Co., Milwaukee, Wis.) per liter and powdered rat chow; (5) monocrotaline plus Captopril, as above; (6) monocrotaline plus penicillamine, as above. Each treatment group consisted of 10 animals. Water and food consumption were measured periodically, and no significant differences were noted among the six groups. All powdered feed contained corn oil (20% w/w) to reduce scattering from the bowl, a problem which interfered with measurement of food consumption.

All animals were sacrificed by decapitation after 6 weeks of continuous drug administration. Blood was collected and clotted on ice. Serum was obtained by centrifugation at 4°C, and was stored at −20°C for determination of ACE activity. The thoracic organs were dissected *en bloc,* and the right lung was ligated at the trachea, removed, and divided into the anatomically distinct upper, middle, and lower lobes. The left lung was distended intratracheally with glutaraldehyde–paraformaldehyde fixative at a pressure of 22 cm of water, and was processed for electron microscopy as described previously (7). The left kidney, adrenal and testis, and the entire spleen, liver, and heart were weighed and fixed in 10% neutral buffered Formalin in order to evaluate nonpulmonary effects of monocrotaline, and to monitor for possible Captopril or penicillamine toxicity. Within 1 month, the Formalin-fixed heart was dissected into the right ventricle (RV) and the left ventricle plus septum (LV + S). Right heart enlargement was evaluated on the basis of RV/LV + S weight ratio.

ACE activity. The middle lobe of the right lung was weighed, frozen in liquid N_2, and stored at −20°C. Within 2 weeks after autopsy, the lung and serum samples were thawed, and the lung was homogenized. ACE activity in the lung homogenate and blood serum was determined by the spectrophotometric method of Cushman and Cheung (18), using the synthetic substrate hippuryl-L-histidyl-L-leucine (Sigma Chemical Co., St. Louis, Mo.). Protein concentration was determined by the biuret method, using bovine serum albumin as a standard. ACE activity was expressed as mU/lobe, mU/mg wet wt, or mU/mg protein, as described previously (19).

PLA activity. The upper lobe of the right lung was frozen in liquid N_2 and stored at −20°C. Within 2 weeks after autopsy, the samples were thawed, and PLA activity was determined by the fibrin plate lysis method of Astrup and Albrechtsen (20) as described previously (21). PLA activity was expressed as area (mm^2) of the fibrin plate lysed under standard *in vitro* conditions.

Hydroxyproline (HP) content. The lower lobe of the right lung was weighed, frozen in liquid N_2, and stored at −70°C. Within 1 month after autopsy, the samples were thawed and pulverized in liquid N_2. Lipids were extracted from the pulverized tissue as described previously (17). The defatted tissue was dehydrated in a desiccator at room temperature for 48 hr, then HP content was determined by the procedure of Stegemann and Stalder (22). Data were expressed as microgram HP/lobe, microgram HP per gram wet weight, or microgram HP per gram fat-free dry weight.

Two-tailed Student's t test was used to determine the significance of differences between group means (23). Significance was accepted when $P < 0.05$.

Results. *Body and organ weights.* Monocrotaline-treated rats failed to gain weight

TABLE I. BODY AND ORGAN WEIGHTS IN MONOCROTALINE-TREATED RATS[a]

Endpoint	Control	Captopril (Cap)	Penicillamine (Pen)	Monocrotaline (Mono)	Mono + Cap	Mono + Pen
Body wt (g)	414 ± 5[b]	386 ± 9	443 ± 13	367 ± 6*	344 ± 4*	367 ± 8*
Kidney wt (mg/g body wt)	3.40 ± 0.10	3.59 ± 0.17	3.38 ± 0.12	3.56 ± 0.09	3.89 ± 0.12	3.50 ± 0.10
Adrenal wt (mg/100 g body wt)	6.74 ± 0.23	6.95 ± 0.29	6.41 ± 0.30	7.78 ± 0.30	8.08 ± 0.33	8.21 ± 0.80
Testis wt (mg/g body wt)	3.60 ± 0.09	3.84 ± 0.17	3.50 ± 0.11	4.08 ± 0.14	3.97 ± 0.55	4.05 ± 0.07
Spleen wt (mg/g body wt)	1.84 ± 0.04	1.84 ± 0.10	1.91 ± 0.08	2.32 ± 0.04*	2.38 ± 0.10*	2.23 ± 0.11*
Liver wt (mg/g body wt)	37.8 ± 0.8	36.1 ± 1.5	39.4 ± 0.9	39.6 ± 1.0	40.3 ± 0.9	41.1 ± 1.3
Heart wt (mg/g body wt)	3.32 ± 0.07	2.88 ± 0.11*	3.31 ± 0.07	4.07 ± 0.12*	3.60 ± 0.21	3.78 ± 0.18
Rt. ventricle wt. (RV)[c]	142 ± 4	125 ± 4*	153 ± 6	220 ± 12*	168 ± 22	193 ± 14*
L. ventricle + septum (LV + S)[c]	650 ± 18	512 ± 20*	732 ± 24	569 ± 15*	524 ± 27*	606 ± 19
RV/LV + S (%)	22.0 ± 0.7	24.7 ± 1.3	21.1 ± 0.9	39.1 ± 2.4*	31.3 ± 2.5*	31.9 ± 2.2*

[a] Animals received 6 weeks of continuous drug administration.
[b] Mean ± SEM; $N = 10$.
[c] Weight (mg) uncorrected for body weight.
* Different from control, $P < 0.05$.

TABLE II. PULMONARY AND SERUM ENZYME ACTIVITY IN MONOCROTALINE-TREATED RATS[a]

Endpoint	Control	Captopril (Cap)	Penicillamine (Pen)	Monocrotaline (Mono)	Mono + Cap	Mono + Pen
Wet weight (mg/RML)[b]	178 ± 5[c]	184 ± 7	194 ± 5	215 ± 15*	251 ± 7*	233 ± 11*
Wet weight (mg/g body wt)	0.43 ± 0.01	0.47 ± 0.02	0.44 ± 0.01	0.60 ± 0.04*	0.72 ± 0.02*	0.64 ± 0.04*
Protein (mg/RML)	21.6 ± 1.4	24.7 ± 2.2	25.5 ± 1.5	32.7 ± 2.4*	32.2 ± 3.0*	40.4 ± 4.1*
Protein (mg/mg wet wt)	121 ± 7	134 ± 10	132 ± 9	156 ± 14*	128 ± 10	174 ± 18*
ACE activity (mU/RML)	673 ± 43	798 ± 56	790 ± 45	508 ± 55*	523 ± 28*	625 ± 51
ACE activity (mU/mg wet wt)	3.78 ± 0.24	4.32 ± 0.22	4.06 ± 0.18	2.40 ± 0.24*	2.08 ± 0.09*	2.73 ± 0.27*
ACE activity (mU/mg protein)	32.3 ± 2.8	33.4 ± 2.5	31.9 ± 2.9	16.6 ± 2.5*	17.1 ± 1.6*	16.3 ± 1.7*
PLA activity (mm^2 lysed)	96 ± 7	106 ± 6	91 ± 6	54 ± 12*	79 ± 5	90 ± 6
Serum protein (mg/ml)	73.9 ± 2.3	74.1 ± 2.2	74.8 ± 1.8	72.8 ± 1.5	74.6 ± 1.5	74.5 ± 2.7
Serum ACE (mU/ml)	60.8 ± 2.5	87.8 ± 1.8*	58.6 ± 3.4	54.6 ± 5.3	84.1 ± 1.3*	60.7 ± 4.1

[a] Animals received 6 weeks of continuous drug administration.
[b] ACE activity was determined in the middle lobe of the right lung (RML).
[c] Mean ± SEM; $N = 10$.
* Different from control, $P < 0.05$.

normally, and after 6 weeks of treatment were significantly smaller than controls. Neither Captopril nor penicillamine influenced this monocrotaline effect on weight gain. Animals receiving monocrotaline alone also developed cardiomegaly, right ventricular hypertrophy, and a 75% increase in RV/LV + S weight ratio. These cardiac manifestations of pulmonary hypertension were significantly ameliorated by concomitant Captopril and penicillamine. Both modifying agents approximately halved the monocrotaline-induced increase in RV/LV + S (Table I). All monocrotaline-treated groups exhibited significant splenomegaly, however. The slight but nonsignificant increases in kidney, adrenal, testis and liver weight relative to body weight observed in all groups receiving monocrotaline were due largely to decreased body weight rather than to increased organ weight. Rats given Captopril alone exhibited a significant reduction in heart weight relative to body weight, although their RV/LV + S was normal. Animals receiving penicillamine alone did not differ significantly from controls in either body or organ weight.

ACE activity. Animals receiving either Captopril or penicillamine alone did not differ from control with respect to pulmonary wet weight, protein content, or ACE activity. In contrast, monocrotaline-treated rats exhibited a significant increase in wet weight and protein content of the right mid lung, and a significant decrease in lung ACE activity. The reduction in ACE activity was observed whether the data were expressed per right mid lobe, or on the basis of wet weight or protein content. Concomitant Captopril or penicillamine had no effect on this monocrotaline-induced decrease in lung ACE activity (Table II). Serum ACE activity was increased significantly in animals receiving Captopril (with or without monocrotaline), but was normal in all other groups.

PLA activity. Rats receiving monocrotaline only exhibited approximately a 50% reduction in PLA activity in the right upper lung. Concomitant Captopril administration partially prevented this monocrotaline effect, and concomitant penicillamine completely reversed it (Table II). Neither modifying agent alone had a significant effect on lung PLA activity.

Hydroxyproline content. Captopril or penicillamine alone had no effect on lung wet weight, fat-free dry weight, or hydroxyproline content or concentration. Monocrotaline, in contrast, caused significant increases in lung wet weight and hydroxyproline content. Neither Captopril nor penicillamine influenced this monocrotaline-induced weight change, and both agents caused a slight but nonsignificant reduction in hydroxyproline content. When expressed on the basis of wet weight or dry weight, however, hydroxyproline concentration increased significantly in monocrotaline-treated lung, and concomitant Captopril or penicillamine diminished these increases (Fig. 1). In fact, rats receiving monocrotaline plus Captopril exhibited lung hydroxyproline concentrations which were

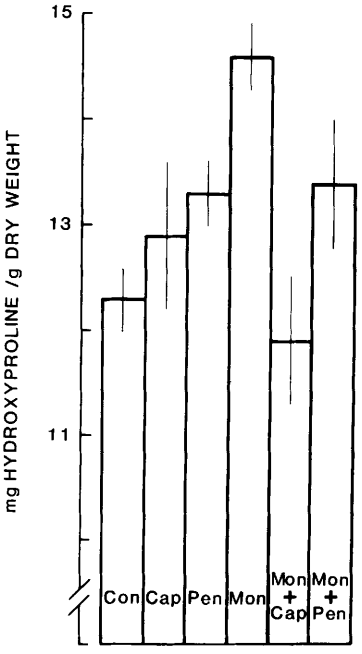

FIG. 1. Hydroxyproline (collagen) concentration in the right lower lung of rats receiving 6 weeks of continuous drug administration. Treatment groups were control (Con), no drug; Captopril (Cap), 60 mg/kg/day, p.o.; D-penicillamine (Pen), 30 mg/kg/day, p.o.; monocrotaline (Mon), 2.4 mg/kg/day, p.o.; monocrotaline plus Captopril (Mon + Cap), as above; or monocrotaline plus penicillamine (Mon + Pen), as above. Hydroxyproline (HP) concentration was determined by the spectrophotometric method of Stegemann and Stalder (22), and was expressed as mg HP/g dry wt. Mean ± SEM; $N = 10$ per group.

TABLE III. PULMONARY HYDROXYPROLINE (HP) CONTENT IN MONOCROTALINE-TREATED RATS[a]

Endpoint	Control	Captopril (Cap)	Penicillamine (Pen)	Monocrotaline (Mono)	Mono + Cap	Mono + Pen
Wet weight (mg/RLL)[b]	401 ± 8[c]	387 ± 14	388 ± 12	434 ± 15	536 ± 16*	474 ± 21*
Wet weight (mg/g body wt)	0.96 ± 0.02	0.99 ± 0.04	0.88 ± 0.01	1.21 ± 0.05*	1.55 ± 0.05*	1.30 ± 0.07*
Fat-free dry wt (mg/RLL)	60.4 ± 1.0	56.9 ± 1.4	55.3 ± 2.1	59.4 ± 2.3	70.1 ± 2.5*	63.4 ± 5.3
Hydroxyproline (μg HP/RLL)	740 ± 13	769 ± 39	750 ± 43	868 ± 35*	828 ± 30*	844 ± 68*
Hydroxyproline (mg HP/mg wet)	1.81 ± 0.05	1.83 ± 0.08	1.89 ± 0.06	2.01 ± 0.03*	1.55 ± 0.06	1.78 ± 0.07
Hydroxyproline (mg HP/g dry)	12.3 ± 0.3	12.9 ± 0.7	13.3 ± 0.3	14.6 ± 0.3*	11.9 ± 0.6	13.4 ± 0.6

[a] Animals received 6 weeks of continuous drug administration.
[b] Hydroxyproline (HP) content was determined in the lower lobe of the right lung (RLL).
[c] Mean ± SEM; $N = 10$.
* Different from control, $P < 0.05$.

slightly lower than those observed in the control group (Table III).

Ultrastructure. Electron micrographs of monocrotaline-treated rat lung revealed inflammatory and hemorrhagic reactions, endothelial degeneration, hypercellularity, muscularization of the arteries and arterioles, and interstitial collagen accumulation (Fig. 2). These monocrotaline-induced changes were less striking in animals receiving concomitant Captopril or penicillamine. The lungs of rats receiving the modifying agents only were indistinguishable from controls.

Discussion. These data are the first to demonstrate that the ACE inhibitor Captopril ameliorates experimental pulmonary fibrosis. The fact that concomitant Captopril administration blocks the increase in hydroxyproline (collagen) concentration in monocrotaline-treated rat lung raises the possibility of a new and previously unsuspected therapeutic application for ACE inhibitors as antifibrotic agents. Electron micrographs confirm the presence of diffuse interstitial fibrosis in monocrotaline-treated lung (Fig. 2). The present data demonstrate that D-penicillamine, an inhibitor of collagen maturation, also ameliorates monocrotaline-induced pulmonary fibrosis in rats. Penicillamine, known to moderate radiation-induced lung fibrosis in rodents (17, 24), thus is shown to be effective in a second model of pulmonary injury.

The mechanism(s) by which Captopril and penicillamine inhibit monocrotaline-induced pulmonary fibrosis is not clear from the present data, and will be the subject of further investigation in our laboratory. The antifibrotic effect of both Captopril and penicillamine is accompanied by partial prevention of monocrotaline-induced cardiomegaly, right heart enlargement, and pulmonary arterial muscularization, indicative of protection against the development of pulmonary hypertension. The antifibrotic effect of both modifying agents also is accompanied by amelioration of the monocrotaline-induced decrease in lung PLA activity, indicative of sparing against endothelial injury. On the other hand, the second index of pulmonary endothelial function examined in the present study, i.e., ACE activity, also was suppressed by monocrotaline, and was not spared by

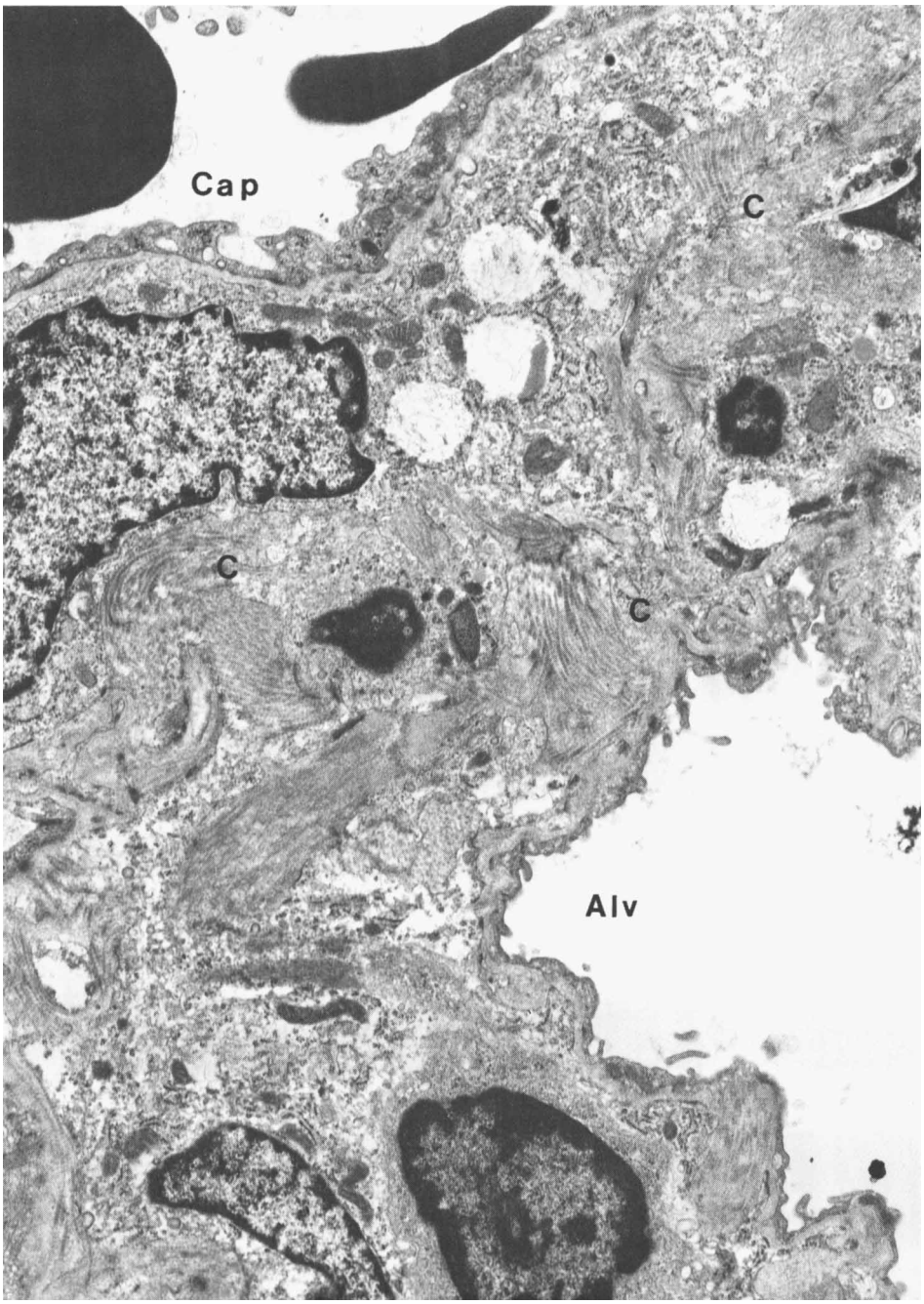

FIG. 2. Interstitial pulmonary fibrosis in a monocrotaline-treated rat. Note the accumulation of collagen fibers (C) in the septum between the alveolar space (Alv) and the blood capillary (Cap). Uranyl acetate and lead citrate stain; ×8700.

concomitant Captopril or penicillamine. Thus the beneficial effect of both modifiers is associated with improvement in some but not all endothelial metabolic activities, implying selective rather than generalized sparing of endothelial function. The fact that both Cap-

topril and penicillamine ameliorate monocrotaline-induced right ventricular hypertrophy and pulmonary fibrosis without influencing lung ACE activity suggests that the latter may not play a crucial role in the pathogenesis of monocrotaline injury in the lung. Thus ACE inhibitors, when protective against experimental pulmonary injury (12–15), may operate via pathways other than inhibition of ACE activity. For example, the ACE inhibitor Teprotide (SQ20881) has been reported to inhibit protein synthesis in the pulmonary artery of chronically hypoxic rats with pulmonary hypertension (25).

The ability of Captopril and penicillamine to ameliorate the monocrotaline-induced suppression in PLA activity may be important to their action as antifibrotics. PLA is a major factor in tissue fibrinolytic activity, and defective fibrinolysis has been proposed as a key element in the development of *radiation*-induced tissue fibrosis (26). According to this hypothesis, ionizing radiation produces vascular endothelial injury, manifest as increased permeability and decreased PLA activity. Both phenomena are well-documented radiation responses of the vasculature (26). Increased permeability leads to the extravasation of fibrinogen and clotting factors, and to the formation of interstitial fibrin. Fibrin strands persist due to defective PLA activity, and serve as a chronic stimulus to fibroblast accumulation and collagen synthesis. It is perhaps not coincidental that the antifibrotic effect of penicillamine in irradiated rat lung (17) is accompanied by amelioration of suppressed PLA activity, with much less effect on suppressed ACE activity (27).

In conclusion, the present study demonstrates that both Captopril and penicillamine exhibit antifibrotic activity in monocrotaline-treated rat lung. While it is feasible to postulate that penicillamine acts via its ability to inhibit collagen crosslinking and maturation (16), such a mechanism has not been considered previously for ACE inhibitors. Alternatively, both agents may act via their ability to spare the pulmonary endothelium against monocrotaline injury. This therapeutic pathway might include preservation of PLA activity, but does not appear to involve ACE activity.

This study was supported in part by PHS Grant HL25106, awarded by the National Heart, Lung, and Blood Institute, DHHS, and by a research grant from Christ Hospital, Oak Lawn, Illinois. The excellent technical assistance provided by Moyra Dunne, Joann Hinz, Gerald Jones, and Chris Granack is greatly appreciated. The authors thank E. R. Squibb and Sons, Princeton, New Jersey, for their generous supply of Captopril.

1. Kay JM, Heath D. *Crotalaria spectabilis:* The Pulmonary Hypertension Plant. Springfield, Ill., Thomas, 1969.
2. Kay JM, Smith P, Heath D. Electron microscopy of *Crotalaria* pulmonary hypertension. Thorax **24**:511–526, 1969.
3. Butler WH. An ultrastructural study of the pulmonary lesion induced by pyrrole derivatives of the pyrrolizidine alkaloids. J Pathol **102**:15–19, 1970.
4. McLean EK. The toxic actions of pyrrolizidine (*Senecio*) alkaloids. Pharmacol Rev **22**:429–483, 1970.
5. Meyrick B, Reid L. Development of pulmonary arterial changes in rats fed *Crotalaria spectabilis.* Amer J Pathol **94**:37–50, 1979.
6. Meyrick B, Gamble W, Reid L. Development of *Crotalaria* pulmonary hypertension: Hemodynamic and structural study. Amer J Physiol **239**:H692–H702, 1980.
7. Molteni A, Ward WF, Ts'ao C, Port CD, Solliday NH. Monocrotaline-induced pulmonary endothelial dysfunction in rats. Proc Soc Exp Biol Med **176**:88–94, 1984.
8. 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.
9. Keane PM, Kay JM, Suyama KL, Gauthier D, Andrew K. Lung angiotensin converting enzyme activity in rats with pulmonary hypertension. Thorax **37**:198–204, 1982.
10. Huxtable R, Ciaramitaro D, Eisenstein D. The effect of a pyrrolizidine alkaloid, monocrotaline, and pyrrole, dehydroretronecine, on the biochemical functions of the pulmonary endothelium. Mol Pharmacol **14**:1189–1203, 1978.
11. Hilliker KS, Bell TG, Roth RA. Pneumotoxicity and thrombocytopenia after single injection of monocrotaline. Amer J Physiol **242**:H573–H579, 1982.
12. Molteni A, Solliday NH, Port CD, Ward WF. Partial prevention of monocrotaline-induced pulmonary arterial changes by the angiotensin-1-converting enzyme (A-1-CE) inhibitor SQ14225. Fed Proc **41**:451 (Abstract), 1982.
13. Molteni A, Solliday NH, Ts'ao C, Ward WF. Mono-

crotaline-induced pulmonary arterial changes in rats: Amelioration by the angiotensin converting enzyme inhibitor CL242817. Fed Proc **43**:784 (Abstract), 1984.

14. Zakheim RM, Mattioli L, Molteni A, Mullis KB, Bartley J. Prevention of pulmonary vascular changes of chronic alveolar hypoxia by inhibition of angiotensin-1-converting enzyme in the rat. Lab Invest **33**:57–61, 1975.
15. Rabinovitch M, Gamble WJ, Williams G, Reid L. SQ14225 converting enzyme inhibitor diminishes pulmonary artery hypertension secondary to chronic hypoxia in rats. Fed Proc **39**:765 (Abstract), 1980.
16. Nimni ME, Deshmukh D, Gerth N. Collagen defect induced by penicillamine. Nature (New Biol) **240**: 220–221, 1972.
17. Ward WF, Shih-Hoellwarth A, Tuttle RD. Collagen accumulation in irradiated rat lung: Modification by D-penicillamine. Radiology **146**:533–537, 1983.
18. Cushman DW, Cheung HS. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem Pharmacol **20**:1637–1648, 1971.
19. Ward WF, Solliday NH, Molteni A, Port CD. Radiation injury in rat lung. II. Angiotensin converting enzyme activity. Radiat Res **96**:294–300, 1983.
20. Astrup T, Albrechtsen OK. Estimation of the plasminogen activator and the trypsin inhibitor in animal and human tissues. Scand J Clin Lab Invest **9**:233–243, 1957.
21. Ts'ao C, Ward WF, Port CD. Radiation injury in rat lung. III. Plasminogen activator and fibrinolytic inhibitor activities. Radiat Res **96**:301–308, 1983.
22. Stegemann H, Stalder K. Determination of hydroxyproline. Clin Chim Acta **18**:267–273, 1967.
23. Dixon WJ, Massey FJ Jr. Introduction to Statistical Analysis, 2nd ed. New York, McGraw–Hill, 1957.
24. Ward WF, Shih-Hoellwarth A, Pearlman HG, Kepka AG. Whole-thorax radiation lethality in penicillamine-treated mice. Radiat Res **90**:321–329, 1982.
25. McKenzie JC, Hung K, Mattioli L, Klein RM. Reduction in hypertension-induced protein synthesis in the rat pulmonary trunk after treatment with Teprotide (SQ20881). Proc Soc Exp Biol Med **177**: 377–382, 1984.
26. Law MP. Radiation-induced vascular injury and its relation to late effects in normal tissues. Adv Radiat Biol **9**:37–73, 1981.
27. Ward WF, Molteni A, Ts'ao C, Solliday NH. Radiation injury in rat lung. IV. Modification by D-penicillamine. Radiat Res **98**:397–406, 1984.

Received January 22, 1985. P.S.E.B.M. 1985, Vol. 180.
Accepted May 2, 1985.