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Antifibrotic effect of captopril and enalapril on paraquat-induced lung fibrosis in rats

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ABSTRACT: Although different treatment modalities have been implemented for pulmonary fibrosis, the results have not been promising and these conditions have been considered untreatable and irreversible. Thus, a plethora of new drugs has been tried for the control of this condition in recent years. This study examined the effects of two angiotensin-converting enzyme inhibitors, captopril and enalapril, on paraquat-induced pulmonary fibrosis in rats, through biochemical and histopathological parameters. Male albino Wistar rats were divided into eight groups (n = 4–5 each), including control, paraquat, captopril alone, captopril treatment and pre-treatment, enalapril alone, enalapril treatment and pre-treatment. After 21 days of treatment, the lungs were removed and the levels of hydroxyproline, glutathione and lipid peroxidation were determined. Angiotensin-converting enzyme inhibitors showed no effect on glutathione and lipid peroxidation. The results also demonstrated that captopril and enalapril improved pulmonary fibrosis as shown by histopathology, as well as a decreased content of hydroxyproline ($P < 0.001$) in the lung tissue. In conclusion, the present findings suggest that the antifibrotic effect of these drugs may be related to the inhibition of angiotensin-converting enzyme. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS: pulmonary fibrosis; paraquat; angiotensin-converting enzyme inhibitors; hydroxyproline; rat

Introduction

Pulmonary fibrosis is a progressive disease for which no effective therapy has been proposed. Current treatments including glucocorticoids and cytotoxic drugs have been aimed at preventing the progression of the disease (Green, 2002). Although the etiology of pulmonary fibrosis is most probably diverse in different individuals, the pathogenesis seems to be the same (Fonseca *et al*., 1999; Marshall *et al*., 1997; Green, 2002). The lesion is characterized by excessive formation of collagen fibers in the extracellular matrix (ECM) of the interstitium of the alveolar walls that leads to reduced expansibility, vital capacity and eventually impaired gas exchange (Franklin, 1997). Death usually occurs due to pulmonary failure. The usual 5 year survival rate is about 50% (Lasky and Ortiz, 2001; Mason *et al*., 1999; Ozyurt *et al*., 2004; Garantziotis *et al*., 2004; Pardo *et al*., 2003; Ward and Hunninghake, 1998).

Angiotensin-converting enzyme (ACE) inhibitors have been used effectively in the treatment of several human diseases including hypertension, congestive heart failure, coronary artery diseases and diabetic nephropathy (Brown and Vaughan, 1998). The therapeutic effect of these drugs is mostly by modulation of the renin-angiotensin system (RAS) (Brown and Vaughan, 1998).

Recent findings also indicate that these agents, mainly captopril, have beneficial effects on animal models of pulmonary fibrosis (Wang *et al*., 2000; Candan and Alagozlu, 2001; Ward *et al*., 1990; Ghazi-Khansari *et al*., 2005).

Although the pharmacological mechanisms of ACE inhibitors are not fully understood, various studies suggest that these agents may effect RAS, inhibition of angiotensin II, stimulation of prostaglandin and bradykinin, and have an antioxidant effect through free radical scavenger action (Tsukada *et al*., 2006; Molteni *et al*., 2001; Demedts *et al*., 2005; Chopra *et al*., 1992; Costa *et al*., 2002).

Paraquat is a broad spectrum contact herbicide that is known to cause progressive pulmonary fibrosis in human and animals (Rose *et al*., 1974; Forman *et al*., 1982; Hettiarachchi and Fernando, 1988). In most instances, death is caused by impairment of the pulmonary function

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which occurs characteristically from several days to 3 weeks after exposure to the toxin (Copland *et al*., 1974; Murray and Gibson, 1972). Paraquat is also a well-known generator of free radicals and a fibrosis inducing agent *in vivo* (Darr *et al*., 1993; Masafumi and Okuyama, 1994; Melchiorri *et al*., 1996).

The aim of this study was to assess and compare the anti-fibrotic effect of two ACE inhibitors, captopril which has a sulfhydryl (SH or thiol) moiety and enalapril which contains a non-sulfhydryl radical in its chemical structure, on paraquat-induced pulmonary fibrosis in rats.

Materials and Methods

Chemicals

Paraquat (methyl viologen), captopril and enalapril were obtained from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals were purchased from Merck (Germany).

Animals

Male albino Wistar rats weighing 150–300 g were received from vivarian section of the Department of Pharmacology, Tehran University of Medical Sciences, and were housed in stainless steel cages covered by wood chips and maintained at room temperature (22 ± 3 °C) under a 12 h light/dark illumination cycle. The animals were allowed free access to tap water and standard diet for the duration of the study. All the procedures in this study were performed in accordance with the guidelines for the Care and Use of Laboratory Animals as adopted by the Ethics Committee of the School of Medicine of Tehran University of Medical Sciences (130/8970, March, 2002).

Experiment Protocols

In order to determine the minimum fibrotic dose of paraquat and the submaximal concentrations of captopril and enalapril (the concentration without any effect on biochemical and histopathological parameters), different concentrations of paraquat (5–20 mg kg^{-1}), captopril (1–100 mg kg^{-1}) and enalapril (0.5–50 mg kg^{-1}) were used. In the present study 20 mg kg^{-1} paraquat, 10 mg kg^{-1} captopril and 5 mg kg^{-1} enalapril were chosen for the rest of the experiments (data not shown).

The rats were randomly divided into eight groups of 4–5 animals each. Group 1 (control) received tap water to drink; group 2 was injected with 20 mg kg^{-1} of paraquat intraperitoneally; group 3 received 10 mg kg^{-1} 24 h^{-1} captopril orally; group 4 received captopril after injection of a single dose of paraquat (20 mg kg^{-1}) intraperitoneally considered as the treatment group of captopril; group 5 received captopril 1 day before injection of a single dose of paraquat (20 mg kg^{-1}) intraperitoneally considered as the pretreatment group of captopril; group 6 received 5 mg kg^{-1} 24 h^{-1} enalapril orally; group 7 received enalapril after injection of a single dose of paraquat (20 mg kg^{-1}) intraperitoneally considered as the treatment group of enalapril, and the group 8 received enalapril 1 day before injection of a single dose of paraquat (20 mg kg^{-1}) intraperitoneally considered as the pretreatment group of enalapril. Paraquat powder was dissolved in distilled water to be prepared for injection. Captopril and enalapril were dissolved in the drinking water of the animals. The water intake was measured daily in each cage, for adjustment of the concentration of the ACE inhibitors to the amount of water consumed. This regimen was maintained during the study. At the end of the treatment period (21 days), the rats were killed by cervical dislocation; the chests were opened immediately for access and examination of the lungs.

After opening the chest, 5 ml 0.9% cold saline solution was injected into the heart. The right lung was removed and was washed two times with cold saline solution and blotted dry. Forty mg of the lung tissue was homogenized in 1 ml of 0.9% cold saline solution and was used for the analysis of glutathione (GSH), lipid peroxidation levels and hydroxyproline content. Briefly, 0.025 ml of the lung tissue homogenate was used for the determination of the hydroxyproline content and the remaining homogenate was mixed with 0.2 ml 20% (w/v) trichloroacetic acid, vortexed and then centrifuged at 6000 rpm for 20 min. The supernatant fraction was used for the determination of the GSH concentration and the sediment fraction was used for the measurement of lipid peroxidation. The left lungs were removed for histopathological studies and then fixed in an adequate amount of 10% neutral buffered formalin solution for 24 h.

Biochemical Analyses

The glutathione level was measured in the supernatant fraction of lung tissue homogenate using 0.04%, 5, 5-dithiobis-[2-nitrobenzoic acid] at 412 nm according to the method of Kuo and Hook (1982). The lipid peroxidation level was measured in the sediment fraction of lung tissue homogenate using thiobarbituric acid at 532 nm and 1,1,3,3, tetraethoxypropane as a standard according to the method of Esterbauer and Cheeseman (1990). The content of collagen in the lung was analysed by measuring the level of hydroxyproline at 550 nm according to the method of Reddy and Enwemeka (1996). The procedure is based on alkaline hydrolysis of the tissue homogenate and subsequent determination of the free hydroxyproline in hydrolysates.

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Histopathological Evaluation

The left lung of the rats was fixed by constant pressure inflation using 10% neutral buffered formalin solution for 24 h. After routine tissue processing, 4 μm thick tissue sections were prepared from the paraffin blocks and were stained by hematoxylin and eosin (H & E) and Masson's trichrome staining methods. One pathologist blind to the study performed all the histological examinations. Histological changes were screened in the H&E stained sections. The presence of fibrosis was evaluated in the Masson's trichrome stained sections more specifically.

Statistical Analysis

The results of the measurements are presented as the mean ± SEM. The difference of the measurements among different groups was analysed by one-way analysis of variance (ANOVA) using the Student-Newman-Keuls multiple comparisons test. A value of $P < 0.05$ was considered significant.

Results

Lung Glutathione (GSH) Content

As shown in Fig. 1, the lung glutathione (GSH) content was significantly decreased in the paraquat group when compared with the control group after 21 days of the treatment (29.3 ± 1.4 μg l^{-1}, $P < 0.05$). Captopril and enalapril failed to prevent the paraquat-induced decreases of GSH.

Lung Lipid Peroxidation Content

There was no significant difference in lung lipid peroxidation content between the control group and the paraquat group after 21 days of the treatment (data not shown).

Lung Hydroxyproline Content

As shown in Fig. 2, the lung hydroxyproline content was significantly increased in the paraquat group when compared with the control group after 21 days of the study (3.46 ± 0.38 mg g^{-1} of tissue, $P < 0.001$). On the other hand, the lung hydroxyproline content was decreased significantly in the treatment and pretreatment groups of captopril when compared with the paraquat group (1.54 ± 0.16 mg g^{-1} of tissue; $P < 0.001$, 1.72 ± 0.16 mg g^{-1} of tissue; $P < 0.001$, respectively). Moreover, in the treatment and pretreatment groups of enalapril, the lung hydroxyproline content decreased significantly when compared with the paraquat group (1.94 ± 0.22 mg g^{-1} of tissue; $P < 0.001$, 1.56 ± 0.19 mg g^{-1} of tissue; $P < 0.001$, respectively).

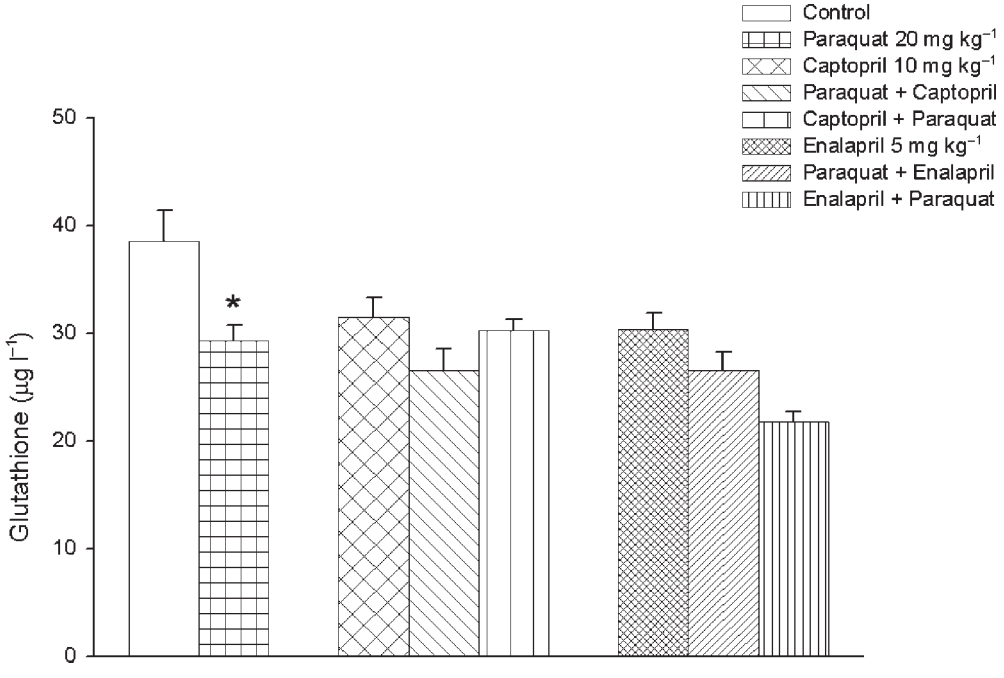

Figure 1. Glutathione levels of lung homogenate in rats at 21 days following treatment with paraquat, captopril and enalapril. * A significant decrease of the paraquat group in comparison with the control group (29.3 ± 1.4 μg l^{-1}, $P < 0.05$)

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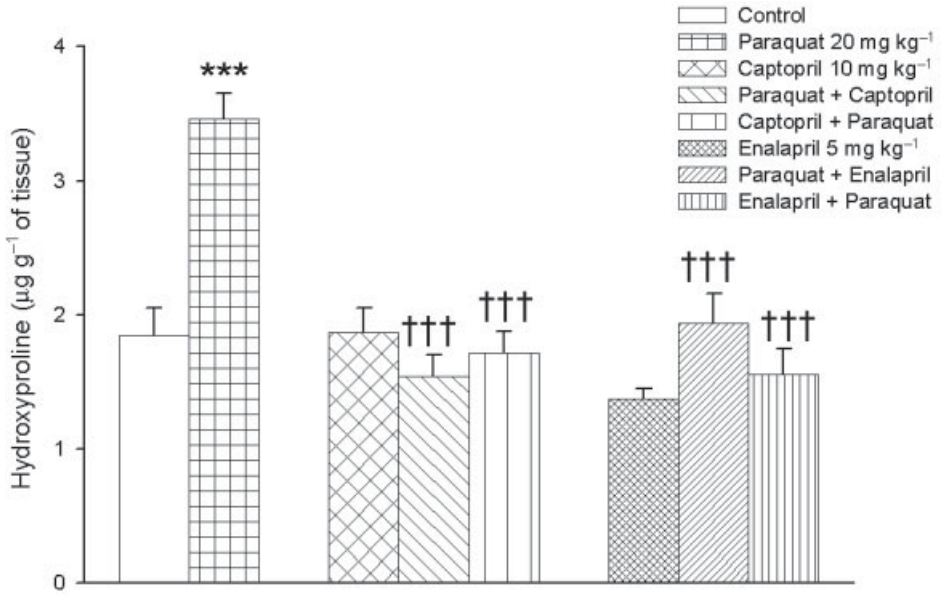

Figure 2. Hydroxyproline content of lung homogenate in rats at 21 days of following treatment with paraquat, captopril and enalapril. *** A significant increase of the paraquat group in comparison with the control group (3.46 ± 0.38 mg g^{-1} of tissue, $P < 0.001$). ††† A significant decrease of the captopril groups in comparison with the paraquat group (1.54 ± 0.16 mg g^{-1} of tissue; $P < 0.001$, 1.72 ± 0.16 mg g^{-1} of tissue; $P < 0.001$, respectively). ††† A significant decrease of the enalapril groups in comparison with the paraquat group (1.94 ± 0.22 mg g^{-1} of tissue; $P < 0.001$, 1.56 ± 0.19 mg g^{-1} of tissue; $P < 0.001$, respectively)

Histopathology

Haematoxylin-eosin and Masson's trichrome stained lung sections were examined by light microscopy. Masson's trichrome staining for collagen in the lungs of the eight groups of rats at 21 days is shown in Fig. 3. Based upon histological observations, no fibrosis was found in the lungs of the rats in captopril and enalapril alone groups

Figure 3. A–H. Comparison of lung size and collagen deposition in lung of control, paraquat, captopril and enalapril treatments rats at 21 days. (A) Control: The air spaces are open. The alveolar septa (yellow arrows) are within normal limits and do not show any inflammation or fibrosis. The red arrow shows a normal airway. The blue collagen fibers seen around the airway are considered a normal finding. The blue arrow shows a normal blood vessel. Masson's trichrome staining (×100); (B) Captopril: The air spaces are open. The alveolar septa (yellow arrows) are within normal limits and do not show any inflammation or fibrosis. The red arrows show a large and a small normal airway. The blue collagen fibers seen around the large airway are considered a normal finding. Masson's trichrome staining (×100); (C) Enalapril: The air spaces are open. The alveolar septa (yellow arrows) are within normal limits and do not show any inflammation or fibrosis. The red arrows show a large and a small normal airway. The blue collagen fibers seen around the large airway are considered a normal finding. Masson's trichrome staining (×100); (D) Paraquat: Many air spaces are filled by inflammatory cells and foamy cells (green arrows). The alveolar septa are thickened due to infiltration of inflammatory cells and deposition of collagen evident by presence of the blue colored fibers (yellow arrow). A small airway is present in this section (red arrow). Masson's trichrome staining (×200); (E) Paraquat + Captopril: The air spaces are open. The alveolar septa (yellow arrows) are within normal limits and do not show any inflammation or fibrosis. The red arrow shows a normal airway. The blue collagen fibers seen around the airway are considered a normal finding. The blue arrow shows a normal blood vessel. Masson's trichrome staining (×100); (F) Captopril + Paraquat: The air spaces are open. The alveolar septa (yellow arrows) are within normal limits and do not show any inflammation or fibrosis. The red arrow shows a normal airway. The blue collagen fibers seen around the airway are considered a normal finding. The blue arrow shows a normal blood vessel. Masson's trichrome staining (×100); (G) Paraquat + Enalapril: The air spaces are open. The alveolar septa (yellow arrows) are within normal limits and do not show any inflammation or fibrosis. The red arrows show two normal airways. The blue collagen fibers seen around the larger airway is considered a normal finding. Masson's trichrome staining (×100); (H) Enalapril + Paraquat: The air spaces are open. The alveolar septa (yellow arrows) are within normal limits and do not show any inflammation or fibrosis. The red arrow shows a normal airway. The blue collagen fibers seen around the airway are considered a normal finding. The blue arrow shows a normal blood vessel. Masson's trichrome staining (×100). This figure is available in colour online at www.interscience.wiley.com/journal/jat

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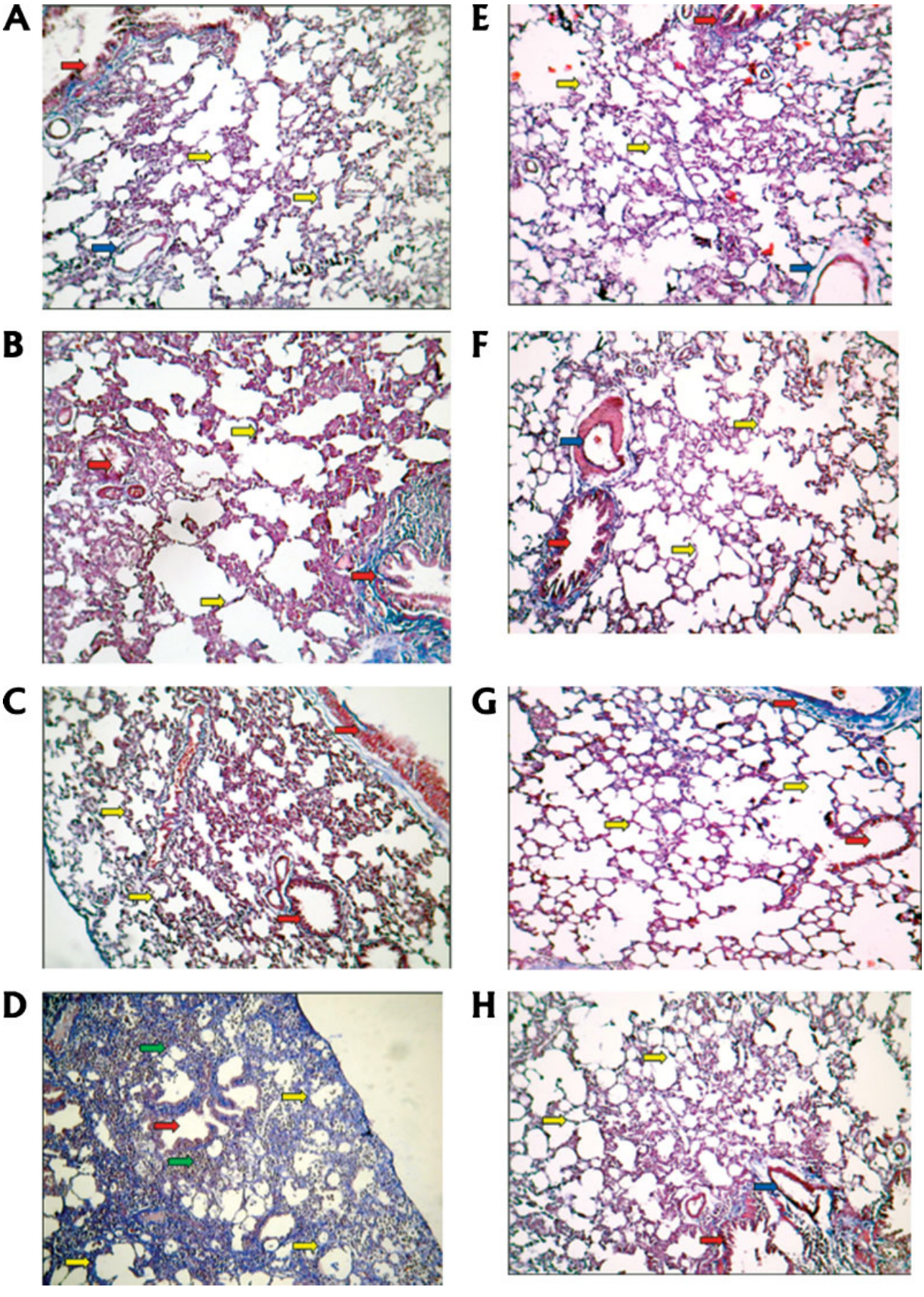

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when compared with the control group (Fig. 3 B, C). Lungs from the paraquat group, however, demonstrated extensive change including focal inflammation with infiltration of lymphocytes and histiocytes in the alveolar spaces and septa and fibrosis leading to increased thickness of the alveolar walls (Fig. 3 D). No fibrosis based upon histological observations was found in the lungs of the rats in the captopril and enalapril treatment and pretreatment animals (groups 4–5, 7–8 respectively) (Fig. 3 E, F, G, H).

Discussion

Current treatments of pulmonary fibrosis have a minimal beneficial effect on the prognosis and many have significant side effects. Therefore, several studies had been undertaken to improve the treatment modalities in this crippling and often fatal condition (Lasky and Ortiz, 2001; Mason *et al*., 1999; Pardo *et al*., 2003; Ward and Hunninghake, 1998). Although the etiology of pulmonary fibrosis is most probably diverse in different individuals, the pathogenesis seems to be the same (Fonseca *et al*., 1999; Marshall *et al*., 1997; Green, 2002). In most studies, the bleomycin-induced pulmonary lesion has been used as a model of fibrosis of lungs in rats (Ozyurt *et al*., 2004; Pardo *et al*., 2003). However, paraquat was used as the fibrogenic agent in this study, because in clinical cases no useful treatment is currently available. Therefore, finding an agent to ameliorate paraquat-induced pulmonary fibrosis could be beneficial.

Angiotensin-converting enzyme inhibitors are known to be able to block the renin–angiotensin system (RAS) and to reduce the systemic blood pressure (Brown and Vaughan, 1998). ACE inhibitors are considered a rather safe group of therapeutic agents with no serious side effects. Thus, there is increasing awareness that the broader pharmacologic properties of ACE inhibitors encompass the ability of antioxidant and antifibrotic in a variety of organ systems (Wang *et al*., 2000; Candan and Alagozlu, 2001; Ward *et al*., 1990; Ghazi-Khansari *et al*., 2005).

The results of several studies performed on tissue damage and repair systems have confirmed the presence of an antifibrotic effect in these agents (Tsukada *et al*., 2006; Molteni *et al*., 2001; Ohishi *et al*., 2001). However, studies for the determination of the effects of ACE inhibitors on pulmonary fibrosis due to paraquat toxicity are scarce.

Two structurally different angiotensin-converting enzyme inhibitors were selected, one with a sulfhydryl (captopril) and the other without a sulfhydryl group (enalapril), to study the progress of pulmonary fibrosis. In this study, the lung glutathione level was decreased at 21 days after treatment with paraquat in which captopril and enalapril had no effect on the paraquat-induced decrease of glutathione. Moreover, paraquat had no effect on lipid peroxidation level of the lung. This finding is in agreement with Kaetsu *et al*. (2001) who showed that the concentration of malondialdehyde in rat lung homogenate did not change after 14 and 28 days exposure of paraquat. Also, previous studies of chronic administration of paraquat showed that paraquat can cause morphological change without an increase of lipid peroxidation (Saunier *et al*., 1982; Kifune *et al*., 1990).

An increase in lung hydroxyproline content, a good index of collagen deposition, was observed 21 days after paraquat treatment correlating well with the histological evidence of fibrosis in most animals in the paraquat group. This finding is in agreement with several other studies that showed an enhancement of collagen synthesis in the lung after exposure to paraquat (Rose *et al*., 1974; Mohammadi-Karakani *et al*., 2006; Virjeyaratnam and Corrin, 1971). In addition, captopril and enalapril could attenuate the pulmonary fibrosis in the treatment and pre-treatment groups of rats. This protective effect was shown by the significant decrease in the pulmonary content of hydroxyproline and histological evaluation of the collagen fiber content by the tissue sections stained by the Masson's trichrome staining method.

These data and the data of our previous study (Mohammadi-Karakani *et al*., 2006) suggest that inhibition of angiotensin-converting enzyme by both non-SH ACE inhibitors and ACE inhibitors containing SH groups is the main pharmacological effective mechanism for inhibition of the fibrosis in paraquat-induced lung injury. Although, several studies have suggested a beneficial effect of antioxidant treatments in the amelioration of pulmonary fibrosis (Lasky and Ortiz, 2001; Demedts *et al*., 2005; Kuwano *et al*., 2001; Ghazi-Khansari *et al*., 2005) and paraquat-induced pulmonary lesions (Suntres, 2002), the results of the lipid peroxidation and glutathione levels of the captopril treated group and the enalapril treated group obtained in our study do not support such findings. It can be concluded that the antifibrotic effect of captopril is most probably not related to the presence of the sulfhydryl group in the chemical structure and hence not related to the antioxidant activity of the molecule. Angiotensin II seems to act as a fibroblast mitogen in the lung and as a mediator of fibroblast proliferation which appears to be linked to the autocrine production of transforming growth factor β (TGF-β) (Ohishi *et al*., 2001; Rocco *et al*., 2001; Marshall *et al*., 2004; Booz *et al*., 1993; Wolf *et al*., 1992; Chen *et al*., 2002; Brilla *et al*., 1993; Carre and Leophone, 1993; Gross and Hunninghake, 2001). Supporting this point of view, several studies showed that TGF-β, a multifunctional cytokine, is the main cytokine involved in the process of fibrosis via the conversion of fibroblasts to myofibroblasts and collagen synthesis (Wolf *et al*., 1992; Brilla *et al*., 1993; Wang *et al*., 2002; Nadrous *et al*., 2005). ACE inhibitors may be helpful in future in therapeutic approaches against the lung fibrosis induced by certain of chemicals including paraquat.

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References

Booz GW, Schorb W, Dostal DE, Conrad KM, Chang KC, Baker KM. 1993. Angiotensin II is a mitogenic in neonatal rat cardiac fibroblast. *Circ. Res.* **72**: 1245–1254.

Brilla CG, Reams GP, Maisch B, Weber KT. 1993. Renin-angiotensin system and myocardial collagen matrix remodeling in hypertensive heart disease. *In vivo* and *in vitro* studies on collagen matrix regulation. *Eur. Heart* 14 (Suppl. J): 57–61.

Brown NJ, Vaughan DE. 1998. Angiotensin-converting enzyme inhibitors. *Circulation* **97**: 1411–1420.

Candan F, Alagozlu H. 2001. Captopril inhibits the pulmonary toxicity of paraquat in rats. *Hum. Exp. Toxicol.* **20**: 637–641.

Carre P, Leophone P. 1993. Cytokines in pulmonary fibrosis. *Rev. Mal. Respir.* **10**: 193–207.

Chen K, Wei Y, Sharp GC, Braley-Mullen H. 2002. Inhibition of TGF-β by anti-TGF-β antibody or lisinopril reduces thyroid fibrosis in granulomatous experimental autoimmune thyroiditis. *J. Immunol.* **169**: 6530–6538.

Chopra M, Beswick H, Clapperton M, Dargie HJ, Smith WE, McMurray J. 1992. Antioxidant effects of angiotensin-converting enzyme (ACE) inhibitors: free radical and oxidant scavenging are sulfhydryl dependent, but lipid peroxidation is inhibited by both sulfhydryl- and nonsulfhydryl-containing ACE inhibitors. *J. Cardiovasc. Pharmacol.* **19**: 330–340.

Copland GM, Kolin A, Shulman HS. 1974. Fatal pulmonary intra-alveolar fibrosis after paraquat ingestion. *N. Engl. J. Med.* **291**: 290–292.

Costa LE, La-Padula P, Lores-Arnaiz S, D'Amico G, Boveris A, Kurnjek ML, Basso N. 2002. Long-term angiotensin II inhibition increases mitochondrial nitric oxide synthase and not antioxidant enzyme activities in rat heart. *J. Hypertens.* **20**: 2487–2494.

Darr D, Combs S, Murad S, Pinnell S. 1993. Studies on the inhibition of collagen synthesis in fibroblasts treated with paraquat. *Arch. Biochem. Biophys.* **306**: 267–271.

Demedts M, Behr J, Buhl R, Costabel U, Dekhuijzen R, Jansen HM, MacNee W, Thomeer M, Wallaert B, Laurent F, Nicholson AG, Verbeken EK, Verschakelen J, Flower CD, Capron F, Petruzzelli S, De Vuyst P, van den Bosch JM, Rodriguez-Becerra E, Corvasce G, Lankhorst I, Sardina M, Montanari M; IFIGENIA Study Group. 2005. High-dose acetylcysteine in idiopathic pulmonary fibrosis. *N. Engl. J. Med.* **353**: 2229–2242.

Esterbauer H, Cheeseman KH. 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods Enzymol.* **186**: 407–421.

Fonseca C, Abraham D, Black CM. 1999. Lung fibrosis. *Springer Semin. Immunopathol* **21**: 453–474.

Forman HJ, Aldrich TK, Posner MA, Fisher AB. 1982. Differential paraquat uptake and redox kinetics of rat granular pneumocytes and alveolar macrophages. *J. Pharmacol. Exp. Ther.* **221**: 428–433.

Franklin TJ. 1997. Therapeutic approaches to organ fibrosis. *Int. J. Biochem. Cell. Biol.* **29**: 79–89.

Garantziotis S, Steele MP, Schwartz DA. 2004. Pulmonary fibrosis: thinking outside of the lung. *J. Clin. Invest.* **114**: 319–321.

Ghazi-Khansari M, Nasiri G, Honarjoo M. 2005. Decreasing the oxidant stress from paraquat in isolated perfused rat lung using captopril and niacin. *Arch. Toxicol.* **79**: 341–345.

Green FH. 2002. Overview of pulmonary fibrosis. *Chest* **122**(Suppl 6): 334S–339S.

Gross TJ, Hunninghake GW. 2001. Idiopathic pulmonary fibrosis. *N. Engl. J. Med.* **345**: 517–525.

Hettiarachchi J, Fernando SS. 1988. Pulmonary fibrosis following paraquat poisoning. *Ceylon. Med. J.* **33**: 141–142.

Kaetsu A, Fukushima T, Inoue S, Lim H, Moriyama M. 2001. Role of heat shock protein 60 (HSP60) on paraquat intoxication. *J. Appl. Toxicol.* **21**: 425–430.

Kifune K, Takatsu A, Shigeta A, Kuniyoshi N. 1990. Scanning electron microscopic study of rat lungs in experimental chronic paraquat poisoning. *Nihon Hoigaku Zasshi* **44**: 302–313 (Abstract).

Kuo CH, Hook JB. 1982. Depletion of renal glutathione content and nephrotoxicity of cephaloridine in rabbit, rats, mice. *Toxicol. Appl. Pharmacol.* **65**: 292–302.

Kuwano K, Hagimoto N, Hara N. 2001. Molecular mechanisms of pulmonary fibrosis and current treatment. *Curr. Mol. Med.* **1**: 551–573.

Lasky JA, Ortiz LA. 2001. Antifibrotic therapy for the treatment of pulmonary fibrosis. *Am. J. Med. Sci.* **332**: 213–218.

Marshall RP, Gohlke P, Chambers RC, Howell DC, Bottoms SE, Unger T, McAnulty RJ, Laurent GJ. 2004. Angiotensin II and the fibroproliferative response to acute lung injury. *Am. J. Physiol. Lung. Cell. Mol. Physiol.* **286**: L156–L164.

Marshall RP, McAnulty RJ, Laurent GJ. 1997. The pathogenesis of pulmonary fibrosis: is there a fibrosis gene? *Int. J. Biochem. Cell. Biol.* **29**: 107–120.

Masafumi T, Okuyama T. 1994. Effect of paraquat on malondialdehyde level in the rat liver microsome. *Arch. Toxicol.* **8**: 187–192.

Mason RJ, Schwarz MI, Hunninghake GW, Musson RA. 1999. NHLBI Workshop Summary. Pharmacological therapy for idiopathic pulmonary fibrosis. Past, present, and future. *Am. J. Respir. Crit. Care Med.* **160**: 1771–1777.

Melchiorri D, Reiter RJ, Sewerynek E, Hara M, Chen L, Nistico G. 1996. Paraquat toxicity and oxidative damage. Reduction by melatonin. *Biochem. Pharmacol.* **51**: 1095–1099.

Mohammadi-Karakani A, Ghazi-Khansari M, Sotoudeh M. 2006. Lisinopril ameliorates paraquat-induced lung fibrosis. *Clin. Chim. Acta* **367**: 170–174.

Molteni A, Moulder JE, Cohen EP, Fish BL, Taylor JM, Veno PA, Wolfe LF, Ward WF. 2001. Prevention of radiation-induced nephropathy and fibrosis in a model of bone marrow transplant by an angiotensin II receptor blocker. *Exp. Biol. Med.* **226**: 1016–1023.

Murray RE, Gibson JE. 1972. A comparative study of paraquat intoxication in rats, guinea pigs and monkeys. *Exp. Mol. Pathol.* **17**: 317–325.

Nadrous HF, Pellikka PA, Krowka MJ, Swanson KL, Chaowalit N, Decker PA, Ryu JH. 2005. The impact of pulmonary hypertension on survival in patients with idiopathic pulmonary fibrosis. *Chest* **128**: 616S–617S.

Ohishi T, Saito H, Tsusaka K, Toda K, Inagaki H, Hamada Y, Kumagai N, Atsukawa K, Ishii H. 2001. Anti-fibrogenic effect of an angiotensin converting enzyme inhibitor on chronic carbon tetrachloride-induced hepatic fibrosis in rats. *Hepatol. Res.* **21**: 147–158.

Ozyurt H, Sogut S, Yildirim Z, Kart L, Iraz M, Armutcu F, Temel I, Ozen S, Uzun A, Akyol O. 2004. Inhibitory effect of caffeic acid phenethyl ester on bleomycine-induced lung fibrosis in rats. *Clin. Chim. Acta* **339**: 65–75.

Pardo A, Ruiz V, Arreola JL, Ramirez R, Cisneros-Lira J, Gaxiola M, Barrios R, Kala SV, Lieberman MW, Selman M. 2003. Bleomycin-induced pulmonary fibrosis is attenuated in gamma-glutamyl transpeptidase-deficient mice. *Am. J. Respir. Crit. Care. Med.* **167**: 925–932.

Reddy GK, Enwemeka CS. 1996. Simplified method for the analysis of hydroxyproline in biological tissue. *Clin. Biochem.* **9**: 225–229.

Rocco PR, Negri EM, Kurtz PM, Vasconcellos FP, Silva GH, Capelozzi VL, Romero PV, Zin WA. 2001. Lung tissue mechanics and extracellular matrix remodeling in acute lung injury. *Am. J. Respir. Crit. Care. Med.* **164**: 1067–1071.

Rose MS, Smith LL, Wyatt I. 1974. Evidence for energy-dependent accumulation of paraquat into rat lung. *Nature* **252**: 314–315.

Saunier C, Schrijen F, Gille JP, Horsky P, Peslin R, Hartemann D, Foliguet B, Lambert H. 1982. Experimental chronic paraquat poisoning. Functional and histopathological pulmonary changes. *Bull. Eur. Physiopathol. Respir.* **18**: 863–876 (Abstract).

Suntres ZE. 2002. Role of antioxidants in paraquat toxicity. *Toxicology* **180**: 65–77.

Tsukada S, Parsons CJ, Rippe RA. 2006. Mechanisms of liver fibrosis. *Clin. Chim. Acta* **364**: 33–60.

Virjeyaratnam GS, Corrin B. 1971. Experimental paraquat poisoning: a histological and electron-optical study of the changes in the lung. *J. Pathol.* **103**: 123–129.

Wang R, Ibarra-Sunga O, Verlinski L, Pick R, Uhal BD. 2000. Abrogation of bleomycin-induced epithelial apoptosis and lung fibrosis by captopril or by a caspase inhibitor. *Am. J. Physiol. Lung. Cell. Mol. Physiol.* **279**: L143–L151.

Wang HD, Yamaya M, Okinaga S, Jia YX, Kamanaka M, Takahashi H, Guo LY, Ohrui T, Sasaki H. 2002. Bilirubin ameliorates bleomycin-induced pulmonary fibrosis in rats. *Am. J. Respir. Crit. Care Med.* **165**: 406–411.

Ward PA, Hunninghake GW. 1998. Lung inflammation and fibrosis. *Am. J. Respir. Crit. Care Med.* **157**: S123–S129.

Ward WF, Molteni A, Ts'ao CH, Hinz JM. 1990. Captopril reduces collagen and mast cell accumulation in irradiated rat lung. *Int. J. Radiat. Oncol. Biol. Phys.* **19**: 1405–1409.

Wolf G, Haberstroh U, Neilson EG. 1992. Angiotensin II stimulates the proliferation and biosynthesis of type I collagen in cultured murine mesangial cells. *Am. J. Pathol.* **140**: 95–107.

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