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Biochemical Pharmacology 64 (2002) 517–525

Biochemical Pharmacology

Effect of pirfenidone against vanadate-induced kidney fibrosis in rats

Mohammed Ali Al-Bayati[a], Yan Xie[a], F. Charles Mohr[b], Solomon B. Margolin[c], Shri N. Giri[a,*]

[a]*Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA 95616, USA*
[b]*Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA*
[c]*Marnac, Inc., Dallas, TX 75231, USA*

Received 21 September 2001; accepted 23 January 2002

Abstract

Renal fibrosis is a complication of kidney injury and can contribute to organ failure. Currently, there are no drugs for the treatment of renal fibrosis. Pirfenidone (PD) has been proven to have antifibrotic effects in animal models of fibrosis. We tested the ability of PD against vanadate-induced kidney fibrosis in rats. The rats were injected subcutaneously with vehicle or vanadate solution (1 mg vanadate/kg/day) for 12 or 16 days to produce varying degrees of kidney fibrosis. The vanadate- and vehicle-treated rats were fed a laboratory diet or the same diet mixed with 0.6% PD *ad lib.* One vanadate-injected group was initially fed the same diet without PD and later switched to the diet containing PD 2 days after the last injection. The rats were killed at 12 and 25 days following the last dose. The changes found in the kidney of vanadate-treated rats included increases in RNA and DNA content and increases in kidney weight. Treatment with PD diminished the vanadate-induced increases in kidney weight and RNA content. The hydroxyproline content of the kidney in vanadate-treated animals was increased significantly ($P \leq 0.05$) from the control level of 1452 μg/kidney to 1765 μg/kidney. Treatment with PD for 37 days caused significant reductions in the vanadate-induced increases in the hydroxyproline level. Similarly, treatment for 41 days also caused significant reductions (1744 μg/kidney) in vanadate-induced increases in the hydroxyproline level (1996 μg/kidney). The histological evaluation revealed that the severity of the lesions in the vanadate-treated group was moderate to severe, and treatment with PD for 41 days decreased the severity to a mild level. In addition, the delayed treatment with PD also minimized the vanadate-induced increases in the collagen content of the kidney. Although it is speculative, PD may potentially be therapeutic in the management of renal fibrosis.

Keywords: Kidney fibrosis; Pirfenidone; Vanadate; Rats

1. Introduction

Kidney fibrosis may develop as a result of chronic infection, obstruction of the ureter by calculi, malignant hypertension, severe diabetic conditions, or chronic exposure to heavy metals such as lead, vanadium, and mercury [1–4]. In addition, idiopathic glomerulosclerosis and renal interstitial fibrosis have been reported in children and adults [5,6]. Kidney fibrosis correlates well with the overall loss of renal function and could contribute to renal failure [7]. At the present time, fibrotic diseases of various organs in humans are treated chronically with high doses of corticosteriods and cytotoxic drugs that have serious systemic adverse effects [8,9].

The antifibrotic effect of PD has been demonstrated in several animal models of fibrosis in different organs. For example, the dietary intake of PD ameliorates bleomycin- and cyclophosphamide-induced lung fibrosis in hamsters and mice [10–13]. Dietary intake of PD reduces collagen accumulation in the remnant kidney with partial nephrectomy and post-obstructive models of kidney fibrosis in rats [14,15]. In addition, treatment with PD and spirnolactone reverses cardiac and renal fibrosis in streptozotocin-diabetic rats [16]. Interestingly, Raghu *et al.* [17] demonstrated the beneficial effects of PD against idiopathic pulmonary fibrosis in an open clinical trial in humans with advanced and end-stage fibrosis.

In humans, loss of kidney functions due to fibrosis usually occurs many years after the onset of diabetes or recurrent infections. The use of animal models for kidney

* Corresponding author. Tel.: +1-530-752-0166; fax: +1-530-752-4698.
E-mail address: sngiri@ucdavis.edu (S.N. Giri).

Abbreviations: PD, pirfenidone; V, vanadate; RD, rat diet; PH, prolyl hydroxylase; ROS, reactive oxygen species; TNF-α, tumor necrosis factor alpha; TGF-β, transforming growth factor beta; and PDGF, platelet-derived growth factor.

0006-2952/02/$ – see front matter
PII: S0006-2952(02)01213-3

fibrosis in the evaluation of drugs for their potential antifibrotic effects may not only help to elucidate the mechanisms of pathogenesis of renal fibrosis, but may also lead to the development of efficacious drugs for the treatment of this debilitating disease in humans. At present, animal models of kidney fibrosis include: (a) ligation of the ureter and partial nephrectomy [14,15], and (b) chronic treatment with cyclosporin A (CsA) in rats on a low-salt diet [18]. The ligation and partial nephrectomy models of kidney fibrosis may alter the systemic and renal physiological processes of the animals, and the CsA model leads to fibrotic changes confined mostly to the medullary region of the kidney [18]. We have demonstrated in a previous study that subcutaneous injections of vanadate in rats at a daily dose of 0.6–0.9 mg/kg for 16 days cause inflammation, cellular hyperplasia, and fibrosis of both the cortical and medullary regions of the kidney when examined at different times during the 25 days following the last injection [1]. These changes appeared to be similar to those found in kidneys of humans at acute, subacute, and chronic stages of inflammation. Consequently, we employed the vanadate-rat model of kidney fibrosis to determine if PD is effective in ameliorating kidney fibrosis. The data presented in this paper indicate that dietary intake of PD prevented the development of vanadate-induced kidney fibrosis, and it also arrested the ongoing and progressive fibroproliferative process of the kidney. It was concluded that PD could be an effective drug for the management of kidney fibrosis resulting from various medical conditions in humans.

2. Materials and methods

2.1. Animals and treatment

A total of 126 male Sprague–Dawley rats (Charles River), 9 weeks old, were used in this study. The average initial weight of these animals was 351 ± 1 g (±SEM). The rats were housed individually in polycarbonate cages in facilities approved by the American Association for the Accreditation of Laboratory Animal Care. These animals were acclimatized to laboratory conditions for 1 week prior to the start of the experiment. A 12 hr/12 hr light/dark cycle was maintained in the housing facilities. The rats were weighed and distributed among the various groups based on their body weight to attain an equal mean weight in all groups. They had free access to water and were fed *ad lib.* either pulverized Rodent Laboratory Chow 5001 (Purina Mills Inc.) or the same pulverized rat chow mixed with 0.6% PD (w/w). PD was donated by Marnac Inc., and all other chemicals were obtained from the Sigma Chemical Co. with purity >99%. The L-[4-^{3}H(N)]proline (specific activity 15–30 Ci/mmol) used to label the procollagen substrate for assaying PH was purchased from New England Nuclear Life Science Products, Inc.

Ammonium metavanadate solution was prepared by dissolving 233 mg of NH_4VO_3 powder in 100 mL of Tris–NaCl buffer (pH 7.4) by stirring for 3 hr. Tris buffer solution was prepared by dissolving 1.21 g of Tris salt [Tris, 2-amino-2-hydroxylmethyl-1,3-propanediol $(HOCH_2)3CNH_2$] in 100 mL of 0.9% sodium chloride solution, and the final pH was adjusted to 7.4 by adding 6 N HCl. Ammonium chloride solution (0.33 mg NH_4^+/mL) was prepared by dissolving 100 mg of ammonium chloride in 100 mL of Tris buffer (pH 7.4). All solutions were filtered through a 0.2-μm pore filter into a sterile container and kept at room temperature until used.

After clipping the hairs between the shoulders of each animal, the vanadate solution in Tris–NaCl buffer (pH 7.3) was injected subcutaneously at 1 mg vanadate/kg/day for 12 days to produce mild to moderate kidney lesions in Study No. 1. The same treatment protocol with vanadate was used for 16 days to produce moderate to severe kidney lesions in Study No. 2. The animals in the control groups were injected subcutaneously with a comparable volume of vehicle solution (ammonium chloride solution, 0.33 mg NH_4^+/kg/day) for the same length of time. The control and the vanadate-treated rats received either the pulverized RD or the same diet mixed with PD. The estimated daily intake of PD in the diet was roughly 500 mg/kg. The animals were acclimatized to the RD or RD + PD for 3 days prior to treatment with vanadate or the vehicle solution.

In each study, the rats were randomly placed into five treatment groups. Two groups were injected with vehicle and three groups were injected with vanadate. One vehicle group was fed the RD, while the other was fed the same diet containing PD. Of the vanadate (V)-injected groups, for the entire period of the study one group was fed the RD (RD + V), while another was fed the RD containing PD (V + PD37 for Study No. 1 and V + PD41 for Study No. 2). The third group of vanadate-injected animals was initially fed the RD and then switched to the same diet containing PD 2 days after the last injection of vanadate and continuing to the end of the study (V + PD23). Rats were weighed daily during the injection periods thereafter, three times per week.

2.2. Sample collections

All rats were killed under pentobarbital anesthesia (70 mg/kg, i.p.) 12 or 25 days following the last vanadate or vehicle injection. Blood for another study was collected from each animal by cardiac puncture at the time of sacrifice. Immediately after collecting blood, the left and right kidneys were excised and weighed individually. The left kidney was cut into four cross-sections using a sharp razor blade and submerged in 10% buffered formalin. These sections were used for histological studies. The right kidney was flash-frozen in liquid nitrogen and stored at −80° until used for the determination of hydroxyproline, protein, RNA and DNA content, and PH activity.

2.3. Histopathology

The fixed kidney cross-sections were embedded in paraffin, and sectioned at 4–6 μm.

Representative sections were stained with Harris' hematoxylin and eosin for histological evaluation and with Masson's trichrome for localization of collagen in the kidneys of the various treatment groups [19]. Two veterinary pathologists read these sections independently (one of the two was not familiar with the treatment protocol).

2.4. Measurement of DNA, RNA, and protein

The frozen right kidneys were first thawed and then homogenized in Tris buffer (0.01 M, pH 7.4). The homogenate was used for the measurement of various biochemical determinants. The protein content of the homogenate was measured by the method of Lowry *et al.* [20], and the DNA and RNA were measured by the method of Wannemacher *et al*. [21].

2.5. Measurement of hydroxyproline

The hydroxyproline level of the kidney homogenates, an index of collagen content, was determined by the colorimetric method of Woessner [22]. Values were expressed as micrograms of hydroxyproline per right kidney.

2.6. Assay of PH activity

The PH assay method was the same as reported previously, and is based on the release of tritiated water from [4-^{3}H]proline-labeled unhydroxylated procollagen substrate prepared *in vitro* using 10-day-old embryonic chick tibiae [23]. About 50 mg of tissue was incubated with the substrate and cofactors for 18 hr. During the reaction, tritiated water is released in stoichiometric proportion to PH activity and is collected by vacuum distillation. The radioactivity of the tritiated water was assessed using a scintillation counter and was a measure of PH activity [24]. The activity was expressed in terms of total dpm released per right kidney in 18 hr.

2.7. Presentation and statistical analysis of data

The biochemical data for all treatment groups are expressed per right kidney in order to avoid artificially lowering the values in the vanadate-treated groups resulting from inflammatory changes in the kidney. The data for each group, presented as means ± SEM, were analyzed by a two-way ANOVA, followed by pairwise comparisons using the Newman–Keuls test. A value of $P \leq 0.05$ was considered to be the minimum level of statistical significance.

3. Results

Since there were no differences in any of the measured biochemical determinants between the two vehicle-injected control groups (one on the RD; and the other on the same diet containing PD), the data from both groups were pooled and depicted as the RD control. The levels of hydroxyproline in kidneys of vanadate-treated rats in both Study No. 1 and Study No. 2 were significantly higher in the RD + V groups than the levels in the corresponding vehicle-treated rats in the control RD groups (see Figs. 1 and 2, respectively). Furthermore, daily injection of vanadate (1 mg/kg) for 16 days in Study No. 2 caused a significantly higher degree of fibrosis than the injection of the same daily dose of vanadate for 12 days when evaluated 25 days following the last injection. A 33% increase in the total amount of injected vanadate in Study No. 2 produced a 65% increase in the hydroxyproline level of the kidney, indicating that the severity of the lesions and the degree of collagen accumulation were functions of the cumulative amount of the administered vanadate (Figs. 1 and 2). It is interesting that the dietary intake of PD for 37 days in Study No. 1 completely prevented the vanadate-

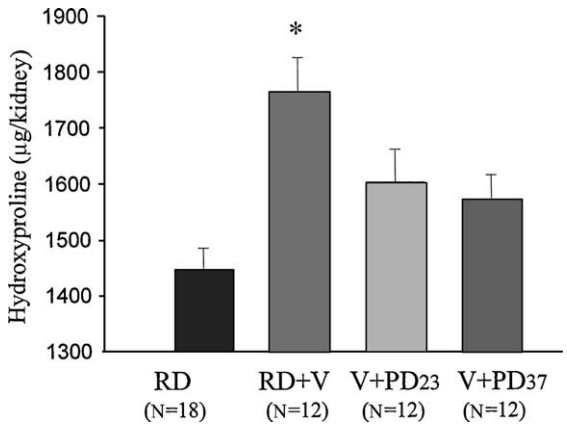

Fig. 1. Effect of PD on vanadate-induced hydroxyproline content of rat kidney in Study No. 1. The animals were fed either a regular rat diet (RD) or the same diet mixed with 0.6% pirfenidone (PD) 3 days prior to subcutaneous injection of vanadate (V) solution (1 mg/kg/day) or an equivalent volume of vehicle for 12 consecutive days. The animals in the various groups received their respective diets for the entire period of the study. The animals were killed at 37 days in each group from the starting day of the experiment, and their right kidneys were processed for hydroxyproline measurement as described under "Section 2" The number of animals in each group is shown in parentheses below each bar, and the treatment groups are indicated along the x-axis. RD: pooled value from two vehicle-injected control groups, one on regular rat diet and the other on the same diet containing PD since there was no difference between these two control groups; RD + V: regular rat diet receiving vanadate; V + PD23: regular rat diet during the period of vanadate injection and switched to the same diet containing PD 2 days after the last vanadate injection, with PD23 referring to 23 days of PD treatment; V + PD37: vanadate-injected animals receiving the rat diet containing PD for the entire 37 days of the study. The values (mean ± SEM) for each group, expressed in μg/kidney, were as follows: RD, 1452 ± 43; RD + V, 1765 ± 53; V + PD23, 1603 ± 53; and V + PD37, 1571 ± 53. Key: (*) significantly higher ($P \leq 0.05$) than all other groups.

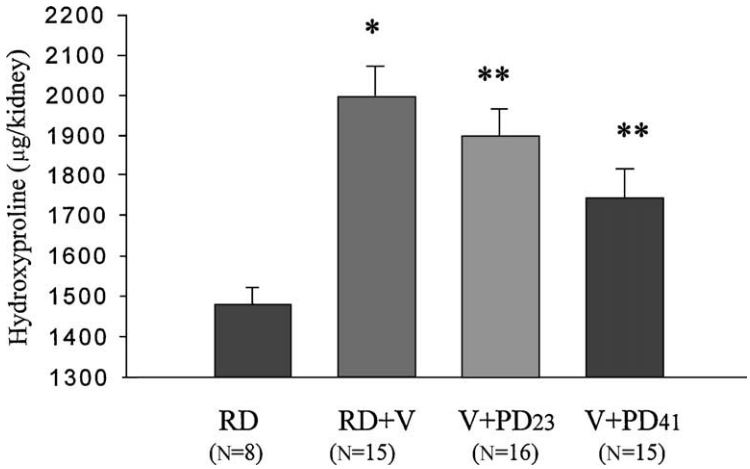

Fig. 2. Effect of PD on vanadate-induced hydroxyproline content of rat kidney in Study No. 2. The experimental protocol for this study was the same as for Study No. 1 except that rats were injected subcutaneously either with an equivalent volume of vehicle or vanadate (V) solution (1 mg V/kg/day) consecutively for 16 days. The animals were killed at 41 days for the measurement of hydroxyproline content of their kidneys. See the legend to Fig. 1 for experimental details and explanation of abbreviations. The values (mean ± SEM) for each group, expressed as μg/kidney, were as follows: RD, 1466 ± 49; RD + V, 1996 ± 72; V + PD23, 1910 ± 69; and V + PD41, 1744 ± 72. Key: (*) significantly higher ($P \leq 0.05$) than RD and V + PD41 groups; and (**) significantly higher ($P \leq 0.05$) than the RD group.

induced increase in the accumulation of collagen in the kidney since there was no significant difference in the hydroxyproline level between the V + PD37 group and the vehicle-control RD group (Fig. 1). Furthermore, PD treatment was also effective in reducing the vanadate-induced increase in collagen deposition in the kidney even when the PD treatment was delayed and begun 2 days after the last injection of vanadate and continued for 23 days (V + PD23 group), since there was no significant difference between the latter and the vehicle control RD group (Fig. 1). The dietary intake of PD also prevented the accumulation of collagen in the kidney in response to a higher cumulative amount of vanadate in Study No. 2 since the hydroxyproline level in the V + PD41 group was significantly less than that of the group treated with vanadate alone (RD + V). However, the level in the V + PD41 group was still significantly higher than in the vehicle control RD group (Fig. 2). Although delayed treatment with PD in the V + PD23 group in Study No. 2 reduced the vanadate-induced increase in collagen content of the kidney compared with the RD + V group, the difference between the two groups was not significant (Fig. 2).

The effects of vanadate treatment with and without dietary intake of PD on the right kidney weight, and the levels of protein, RNA, and DNA are summarized in Table 1 for Study No. 1. Table 2 contains the additional data of PH activity of the kidney for Study No. 2. In Study No.1, there were no significant differences in kidney weights and protein levels among the vehicle control (RD), vanadate-treated (RD + V), and vanadate + PD-treated groups. However, the rats treated with vanadate alone (RD + V) or vanadate + PD (V + PD23 and V + PD37) had significantly higher levels of RNA and DNA than the rats in the vehicle control (RD) group (Table 1). In Study No. 2, kidney weight and the RNA and DNA content were increased significantly in the vanadate-treated (RD + V) group as compared with the vehicle control (RD) group; the vanadate + PD (V + PD23)-treated group also had significantly higher kidney weight and DNA content but not RNA content than the vehicle control (RD) group (Table 2). However, the dietary intake of PD for 41 days (V + PD41) caused significant reductions in the vanadate-induced increases in kidney weight and the level of RNA as compared with the

Table 1
Effects of pirfenidone on vanadate-induced biochemical changes in the right kidney per Study No. 1

Treatment	Measurements per right kidney			
	Weight (g)	Protein (mg)	RNA (mg)	DNA (μg)
RD (N = 18)	1.71 ± 0.05	265 ± 7	29.3 ± 1.1	5265 ± 154
RD + V (N = 12)	1.79 ± 0.05	288 ± 21	33.2 ± 2.5*	6436 ± 503*
V + PD23 (N = 12)	1.82 ± 0.04	279 ± 18	35.7 ± 2.4*	6519 ± 413*
V + PD37 (N = 12)	1.77 ± 0.05	267 ± 8	36.6 ± 1.7*	6472 ± 215*

See "Section 2" for treatment details and the legend of Fig. 1 for an explanation of treatment abbreviations. Vehicle-treated control animals received rat diet (RD) or pirfenidone (PD) mixed with RD. The data from both control groups were pooled since there was no difference between the two groups, and are presented under RD. Values are means ± SEM.

* Significantly higher ($P < 0.05$) than corresponding control RD groups.

Table 2
Effects of pirfenidone on vanadate-induced biochemical changes in the right kidney per Study No. 2

Treatment	Measurements per right kidney				
	Weight (g)	Protein (mg)	RNA (mg)	DNA (μg)	PH activity (dpm × 10^{-5})
RD (N = 8)	1.69 ± 0.06	271 ± 21	36.0 ± 1.1	3362 ± 173	3.3 ± 0.2
RD + V (N = 15)	1.88 ± 0.04*	271 ± 5	40.7 ± 2.6**	5549 ± 218***	3.3 ± 0.5
V + PD23 (N = 16)	1.87 ± 0.03*	269 ± 5	37.4 ± 0.8	5489 ± 235***	3.3 ± 0.2
V + PD41 (N = 15)	1.77 ± 0.05	265 ± 6	34.0 ± 0.9	5531 ± 264***	3.0 ± 0.3

See "Section 2" for treatment details and the legend of Fig. 1 for an explanation of treatment abbreviations. Vehicle-treated control animals received rat diet (RD) or pirfenidone (PD) mixed with RD. The data from both control groups were pooled since there was no difference between the two groups, and are presented under RD.

* Significantly higher ($P < 0.05$) than corresponding control RD and V + PD41 groups.

** Significantly higher ($P < 0.05$) than corresponding RD, V + PD23, and V + PD41 groups.

*** Significantly higher ($P < 0.05$) than corresponding RD groups.

RD + V group, but it failed to affect the DNA content (Table 2). The PH activities of the right kidney in Study No. 2 were just about the same for the various groups (Table 2).

The histopathological changes were characterized in RD control, RD + V, and V + PD groups at 12 and 25 days after the last injection of vanadate in Study No. 2. Two stained paraffin kidney sections were examined per animal. The first section was stained with hematoxylin and eosin for histological evaluation, and the second section was stained with Masson's trichrome for localization of collagen bands in different treatment groups. The severity of the lesions was scored on a scale of 1–5 (1 = normal, 2 = mild damage, 3 = moderate damage, 4 = severe damage, and 5 = excessive damage). Multifocal lesions were observed in the cortex and medulla at 12 and 25 days after the last injection of vanadate in RD + V groups, with the cortex being more affected than the medulla. The changes in the cortex included: deposition of collagen in

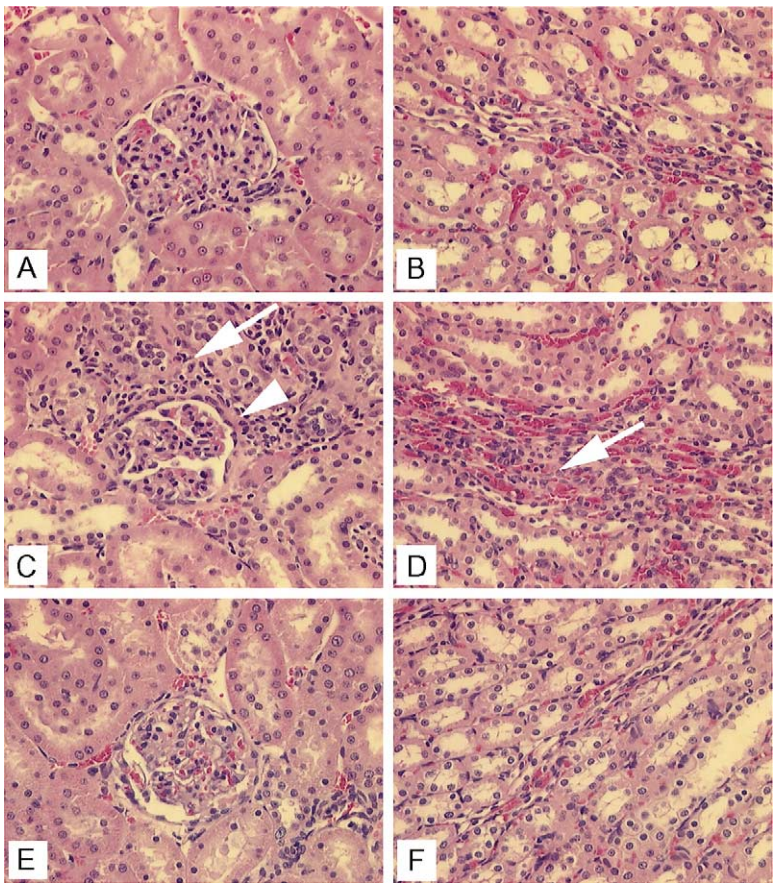

Fig. 3. Representative photomicrographs of rat kidneys stained with hematoxylin and eosin × 400 from rats in RD, RD + V, and V + PD41 groups in Study No. 2. See the legend to Fig. 1 for experimental details and explanation of abbreviations. (A) cortex; (B) medulla from the RD control group showing normal morphological features; (C) cortex; (D) medulla from the RD + V group showing the proliferation of fibroblasts and areas of interstitial fibrosis (arrow) and thickening of Bowman's capsule (arrowhead); (E) cortex; and (F) medulla from the V + PD41 group showing minimal lesions.

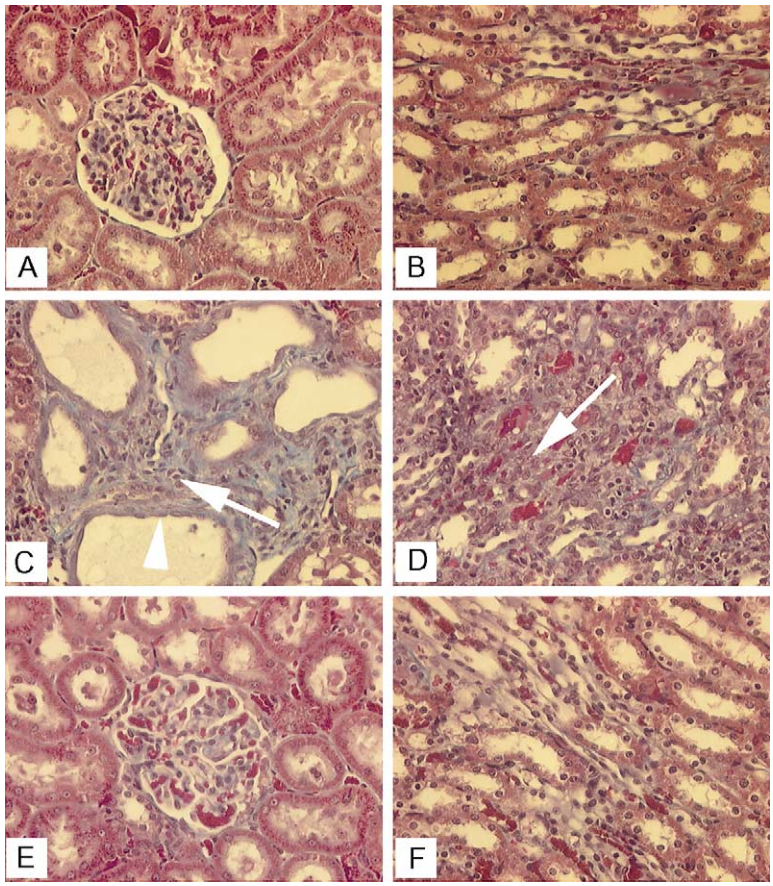

Fig. 4. Representative photomicrographs of rat kidneys stained with Masson's trichrome × 400 from rats in the RD, RD + V, and V + PD41 groups in Study No. 2. See the legend to Fig. 1 for experimental details and explanation of abbreviations. (A) cortex; (B) medulla from the RD control group showing the absence of staining for collagenous materials; (C) cortex; (D) medulla from the RD + V group showing increased deposition of interstitial collagen (arrow) surrounding dilated tubules lined with flattened cells (arrowhead); (E) cortex; and (F) medulla from the V + PD41 group showing little deposition of collagen.

the interstitial spaces surrounding renal tubules and Bowman's capsule and in glomerular tufts. In addition, some of the renal tubules were dilated and lined with flattened epithelium (Figs. 3 and 4). The changes in the medullary region included: hyperplasia of epithelial cells lining the collecting tubules and ascending limb of the loop of Henle and interstitial fibrosis (Figs. 3 and 4). Although these changes were observed in both the RD + V and V + PD groups, they differed in the degree of severity. For example, the severity of the lesions in both the RD + V and V + PD groups was mild to moderate at 12 days as assessed by an average score of 2.13 for both groups. However, at 25 days post-vanadate treatment, the severity of the lesions in the RD + V group increased to moderate to severe levels and the score averaged 3.0. However, treatment with PD in the V + PD41 group decreased the severity of lesions to a very mild degree with an average score of 1.42 (Figs. 3 and 4). The severity of the lesions in vanadate-treated rats that received the PD treatment starting 2 days after the last injection of vanadate and continuing for 23 days was significantly lower, with an average score of 2.0, than the rats in the RD + V group with an average score of 3.0.

4. Discussion

Data presented in this study revealed that vanadate treatment affected both cortical and medullary regions of the kidney and produced moderate to severe damage leading to fibrosis of both regions. The acute stage of inflammation was followed by cortical and medullary fibrosis and medullary epithelial cell hyperplasia. The severity of the lesions and fibrosis was dependent upon the cumulative amount of injected vanadate. The histological and biochemical changes observed in kidneys were similar to the changes reported previously in rats treated with similar doses of vanadate [1]. It is not surprising that the kidney is particularly vulnerable to the toxic effects of vanadate, since it is cleared from the body predominantly via renal excretion in a manner similar to other heavy metals.

The biochemical mechanisms for vanadate-induced kidney damage are not clearly understood. However, it has been hypothesized that the ability of vanadate to interfere with the activities of several key enzymes essential for the survival of cells may play a key role for the injurious

effects of vanadate on kidneys [25,26]. The increased RNA and DNA content of the right kidney and kidney weight in response to vanadate treatment in the RD + V and the V + PD groups, as summarized in Tables 1 and 2, reflect mild to severe pathology in the kidney. It is interesting that PD treatment for 41 days decreased the vanadate-induced increases in kidney weight and its RNA content in the V + PD41 group (Table 2); these effects may be linked with the anti-inflammatory effects of PD. Our attempt to measure the activity of PH was made in the hope of relating the activity of this enzyme with the extent of collagen accumulation in the various treatment groups, since this enzyme is extremely important in the post-translational processing of collagen [27]. Although there are several instances in which the activity of this enzyme has been shown to increase as collagen accumulates in the lungs [28,29], the role of proline hydroxylation in collagen synthesis and subsequent secretion is controversial [30]. It is not clear why the activity of PH remained the same in all groups. However, it is possible that the increase in the activity of this enzyme, at least in the vanadate-treated (RD + V) group, occurred early and then declined to control levels at a much later time when the animals were killed.

It is known that vanadate inflicts oxidative damage on tissue by stimulating the generation of ROS and lipid peroxidation in a dose-dependent manner [31–34]. If vanadate-induced oxidative damage is responsible for the various stages of the inflammation leading to fibrosis, it could explain the beneficial effects of PD in vanadate-induced kidney fibrosis in several ways. First, it has been demonstrated by different investigators that PD *in vitro*-directly scavenges ROS including $O_2^{\bullet -}$, H_2O_2, and $^{\bullet}OH$, resulting in the inhibition of lipid peroxidation in a dose-dependent manner [35,36]. Second, PD is known to have anti-inflammatory effects in the bleomycin-hamster model of acute lung inflammation [37]. Third, PD prevents lipid peroxidation and development of lung fibrosis in different animal models [10–13] as well as the kidney fibrosis caused by ureter ligation and partial nephrectomy [14,15]. These lines of evidence suggest that the inherent ability of PD to scavenge ROS and thereby retard lipid peroxidation and the associated inflammatory changes might be one of the possible mechanisms for its antifibrotic effects against vanadate-induced kidney fibrosis as reported in the present study.

However, other mechanisms including the ability of PD to suppress the production of fibrogenic cytokines such as TNF-α, [38], TGF-β [39], and PDGF [40], as demonstrated by us and other investigators, cannot be ruled out. The overexpression of TGF-β message is considered to be one of the principal causes of kidney [14,15,41] and lung [39,42] fibrosis as demonstrated by various investigators in different animal models as well as in humans suffering from glomerular diseases [43] or idiopathic pulmonary fibrosis [44,45]. In fact, the experimental approaches that down-regulate the overexpression of TGF-β message or neutralize the biological activities of this cytokine have proven to minimize kidney and lung fibrosis [14,15,39,46–48]. The ability of PD to down-regulate the bleomycin-induced overexpression of TGF-β message in lung fibrosis [39] or down-regulate the same in kidney models of fibrosis [14,15] may explain its beneficial effects against vanadate-induced kidney fibrosis as well.

It is unfortunate that the expression of TGF-β message was not investigated in this study. Nevertheless, it is possible that one of the mechanisms for the antifibrotic effect of PD may well reside in its ability to down-regulate the vanadate-induced overexpression of TGF-β message. This is based on the assumption that the expression of fibrogenic cytokine genes including TGF-β occurs in response to activation and translocation of nuclear factor-κB (NF-κB) into the nucleus where this transcription factor binds to the promoter region of genes containing the NF-κB motif and stimulates their expression [49,50]. According to the prevailing theory, NF-κB is an oxidant-sensitive transcription factor [51] and is activated in some cell lines in response to elevated levels of ROS [52,53]. Since vanadate is known to cause oxidative damage by generating ROS in tissues, it is highly likely that it activates NF-κB and thus stimulates the expression of fibrogenic cytokines in the kidney. This theory also explains the antifibrotic effect of PD against vanadate-induced kidney fibrosis since PD is known to be antioxidant by scavanging ROS [35,36], and compounds having antioxidant effects are known to block the ROS-induced activation of NF-κB, thus minimizing the tissue damage in response to oxidants [50,54]. Regardless of the mechanisms, it appears that PD is an effective antifibrotic drug against kidney fibrosis induced by vanadate in the rat model. It is tempting to speculate that PD may have potential in preventing and treating renal fibrosis in humans resulting from various causes such as chronic infections, ureter obstruction, malignant hypertension, diabetic nephropathy, and systemic lupus erythematosus. However, further investigation is warranted to substantiate this speculation.

Acknowledgments

The authors would like to thank Marnac, Inc. for financial support and for the supply of PD for the study.

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