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Effects of high doses of dexamethasone on hemodynamic and immunohistochemical characteristics of acute paraquat intoxication in rat kidneys

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Abstract

Paraquat (1,1′-dimethyl-4,4′-bipyridinium) (PQ), is a nonselective contact herbicide that is highly toxic to humans. The kidney is affected during PQ intoxication. Dexamethasone (Dexa) has anti-inflammatory effects and is used to treat cases of PQ poisoning. We investigated in rat kidney hemodynamic effects and immunohistochemical characteristics of Dexa treatment in acute PQ poisoning. Adult male rats were divided into four groups: 1, untreated control; 2, treated with 100 mg/kg Dexa; 3, treated with 25 mg/kg PQ; 4, treated with PQ + Dexa. Mean arterial pressure (MAP) and heart rate (HR) were recorded during the experimental period (2 h). Tissues were removed after 2 h and immunohistochemistry was performed after 24 h. Paraffin sections of kidney were prepared and anti-cyclo-oxygenase-1 (COX-1), anti-cyclo-oxygenase-2 (COX-2), anti-angiotensin converting enzyme (ACE), anti-aquaporin-1 (AQU-1), anti-vascular cell adhesion molecule (VCAM) primary antibodies were used for immunohistochemical examination. Immunoreactivities were scored as: (1) minimal, (2) weak, (3) mild, (4) moderate, (5) strong and (6) very strong. MAP and HR were measured at 10 min, 20 min, 1 h and 2 h. MAP at 10 and 20 min and 1 h was increased in the Dexa group. HR also was increased in all groups compared to controls at 2 h. Compared to groups 2 and 4, MAP values decreased significantly in group 3 at 1 h. The intensity of all of immunoreactivities was decreased in group 2. In group 3, immunoreactivities of COX-1, COX-2 and ACE were decreased compared to the control and the other groups, whereas AQU-1 and VCAM immunoreactivities were the same as the control group. ACE and VCAM immunoreactivities were decreased in group 4 compared to the control group, while COX-1, COX-2 and AQU-1 immunoreactivities were close to those of the control group. Dexa appears to be useful for treating PQ intoxication.

Key words: angiotensin converting enzyme, aquaporin, cyclo-oxygenase, dexamethasone, hemodynamic effects, immunohistochemistry, kidney, paraquat, rat, vascular cell adhesion molecule

Corticosteroids such as dexamethasone (Dexa) are potent anti-inflammatory agents that are used to treat diseases including rheumatoid arthritis, asthma, systemic lupus erythematosus and atherosclerosis; they also have been used to prevent or reduce the incidence of chronic lung disease (Hench 1949, Neeck et al. 2002, Herrera et al. 2010). Corticosteroids inhibit the inflammatory response by binding to the glucocorticoid receptor. Recent studies, however, suggest that some of the anti-inflammatory effects of corticosteroids occur by non-glucocorticoid receptor-mediated mechanisms (Gude et al. 1990). Corticosteroids also increase blood pressure, alter vascular responses to

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vasoconstrictive and vasodilator agents, and exert some vasoconstrictor activity in the arterioles that regulate vascular tonus (Clifton et al. 2002).

Anti-cyclo-oxygenase (COX) catalyzes rate-limiting oxidative processes during the formation of prostaglandin, tromboxane and prostacyclin from arachidonic acid. COX has two isoforms: COX-1 (72 kDa) is the fixed form and COX-2 (72/74 kDa) is stimulated by growth factors, oncogenes, tumor promoters and pro-inflammatory cytokines. COX-1 can be produced by many tissues, while COX-2 remains inactive in many tissues. Physiologic, pathologic and mitogenic stimulation cause rapid release of large amounts both Cox-1and -2. COX-2 activity is regulated by gene expression and it is specific to the type of cell that secretes it. For example, TGF-β augments COX-2 induction by IL-1 in fibroblasts, whereas it inhibits COX-2 induction in macrophages (Meng et al. 2006).

Leukocyte recruitment into inflamed tissue is a critical step for the development of acute and chronic inflammatory responses (Carlos et al. 1994). This recruitment requires interactions between leukocytes and the vascular endothelium; molecules that are essential for inducing these interactions include chemokines and cellular adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) (Carlos et al. 1994).

Angiotensin converting enzyme (ACE) and ACE_2 are homologous metalloproteases that perform catalytic functions in the renin-angiotensin system (RAS). Angiotensin II is an effector peptide in the RAS; it causes vasoconstriction and has other biological functions including increasing vascular permeability. ACE cleaves angiotensin I to form angiotensin II, whereas ACE_2 reduces angiotensin II levels (Imai 2007).

The water channel in aquaporin (AQU)-1 permits transfer of 3×10^9 water molecules/monomer/sec (Zeidel et al. 1992). Despite its high water permeability, AQU-1 is selective and prevents the transfer of protons. This is important physiologically, because passage of protons through the pore would destroy the proton gradient across the cell membrane that serves as a major energy storage mechanism. The functional roles and medical implications of AQU-1 can be detected in various organs and cells including microvascular endothelial cells, kidney, central nervous system, eye, lacrimal and salivary glands, respiratory apparatus, gastrointestinal tract, hepatobiliary compartments, female and male reproductive system, inner ear and skin. AQU-1 is localized in the apical and basolateral membranes of renal proximal tubules and descending thin limbs of Henle; it is responsible for 75% of water reabsorption from the glomerular filtrate. The expression of AQU-1 is increased in glomerular endothelial cells in a number of kidney diseases and also may play a role in the establishment of salt-sensitive hypertension (Benga 2012).

Paraquat dichloride (1,1′-dimethyl–4,4′-bipyridinium dichloride) (PQ), a nonselective herbicide, was banned in the European Union in 2007 for "not meeting health standards" (http://www.epha.org/a/826), the legal description for severe, often deadly sequelae of PQ toxicity. Many deaths owing to accidental or suicidal ingestion of PQ have been reported. Multiple organs and systems are affected by PQ and structural abnormalities have been observed in lung, kidney, liver and jejunum (Bismuth et al. 1990, Onyeama and Oehme 1984, Samai et al. 2007, Dinis-Oliveira et al. 2009). There is no specific treatment for PQ toxicity. Management of PQ poisoning has been directed toward either decreasing its absorption or enhancing its elimination (Dinis-Oliveira et al. 2006).

Lin et al. (2006) administered repeated doses of methylprednisolone and cyclophosphamide with continuous Dexa administration for patients with severe paraquat poisoning; this regimen decreased mortality. Adjunct therapies for decreasing inflammation also have been beneficial (Chen et al. 2002). Steroid therapy, especially during early exposure, decreased mortality and morbidity and had positive effects on tissue histology and function (Hench 1949). Dexa is a long-acting synthetic glucocorticoid that has been used to treat PQ intoxication (Dinis-Oliveira et al. 2008).

The short term effects of Dexa on hemodynamic and histopathological changes in tissue affected by PQ toxicity have not been examined fully. We investigated the acute hemodynamic effects of Dexa in healthy and PQ treated rats. We also investigated the distributions of ACE, COX-1 and COX-2, AQU-1 and VCAM immunoreactivities in rat kidney as indicators of hemodynamic and histopathological changes.

Material and methods

Animals and experimental design

Our study was approved by the local Animal Ethical Committee of University of Celal Bayar-Manisa. All animals were housed under standard laboratory conditions with a 12 h light/12 h dark cycle at $22 \pm 1°$ C and 50–60% humidity for at least 4 days before starting the experiments. Animals

were allowed access to tap water and rat chow *ad libitum* during the 4 days.

Twenty-eight 240–270 g adult male Sprague-Dawley rats were divided into four groups. Group 1 (controls) received a 1 ml intraperitoneal (i.p.) injection of saline. Group 2 received 100 mg/kg Dexa in 1 ml saline administered i.p. Group 3 received 25 mg/kg PQ administered i.p. Group 4 received PQ and Dexa administered together.

For anesthesia, 50 mg/kg sodium pentobarbital was injected i.p; a maintenance anesthetic dose of 5 mg/kg sodium pentobarbital also was administered i.p. A tracheotomy was performed on the anesthetized animals. All experimental animals breathed spontaneously and no respiratory assistance was required during the experiments. At the beginning of the experiment, breathing frequency was 45–70/min. The left femoral vein was cannulated to administer saline and the left femoral artery was catheterized to record arterial blood pressure. MAP and HR were measured at 10 and 20 min, and at 1 and 2 h. Each experimental period was 2 h for all four groups.

Chemicals

Physiological saline was obtained from Medifleks (Baxter, İstanbul Turkey). Dexa and PQ were obtained from Sigma Chemical Co. (St. Louis, MO). Animals were treated with HPLC grade 98% Dexa dissolved 100 mg/kg in distilled water and prepared fresh before each experiment.

Blood pressure and pulse

Mean arterial pressure (MAP) and heart rate (HR) were recorded for all animals during the experimental period. The blood pressure signals from the left femoral artery were amplified (TBM4-WPI; World Precision Instruments, Tucson, AZ) and recorded digitally (PowerLab/8P; AD Instruments, Castle Hill, NSW, Australia). Heart rate was determined using the pressure signals.

Histochemistry and immunohistochemistry

Animals were sacrificed immediately after experimental treatments. Kidneys were removed after 2 h and fixed in 10% formalin for 24 h at room temperature. Pieces of kidneys were washed overnight with tap water, dehydrated through a graded series of ethanols, cleared in xylene and processed for embedding in paraffin according to routine protocols. Five micrometer sections were cut with a microtome and stained using immunohistochemistry. Briefly, sections were deparaffinized with xylene and rehydrated through a graded ethanol series. Sections then were treated with 0.2% trypsin in Tris buffer (50 mM Tris base and 150 mM NaCl dissolved in deionized water) at 37° C for 15 min to expose antigenic sites. Sections were delineated using a Dako pen (Dako, Glostrup, Denmark) and incubated in 3% H_2O_2 for 5 min to inhibit endogenous peroxidase activity. Sections were incubated with normal serum blocking solution for 1 h, then incubated in a humid chamber for 18 h at 4° C with primary antibodies: anti-ACE (sc-12187- anti-goat polyclonal), anti-COX-1 (sc-1754, anti-goat polyclonal), anti-COX-2 (sc-1747-anti-goat polyclonal), anti-AQU-1 (sc-32737, anti-mouse monoclonal) and anti-VCAM (sc-8304, anti-rabbit polyclonal), all diluted 1:100 and all obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

Sections were incubated with biotinylated IgG, then with streptavidin conjugated to horseradish peroxidase for 30 min each (K0609; Dako LSAB 2 System kit for rabbit and mouse antibodies, and sc-2023; Santa Cruz Biotechnology for goat antibodies) prepared according to kit instructions. Sections finally were incubated with diaminobenzidine hydrochloride (DAB; Dako K3465 kit) prepared according to kit instructions and nuclei were counterstained with Mayer's hematoxylin. All dilutions and washes between steps were performed using phosphate buffered saline, pH 7.4 (PBS). Sections were dehydrated through a graded ethanol series, cleared in xylene, mounted in Entellan (Merck Millipore, Darmstadt, Germany) and analyzed using a Bx40 bright field microscope (Olympus, Tokyo, Japan). Control samples were processed identically, except that incubation with the primary antibody was omitted. Two observers, blinded to experimental information, scored the immunolabeling independently. Three different renal cortex locations were examined at x 400 for each rat. Immunoreactivities were scored as (1) minimal, (2) weak, (3) mild, (4) moderate, (5) strong and (6) very strong.

Statistical analysis

Data are expressed as means ± SE. All data were analyzed using Sigma Stat 2.0 software (SPSS, Chicago, IL). Significant differences were determined using paired and unpaired t-tests. Mann-Whitney U test was used for comparisons between groups. Analysis of variance (ANOVA) as a non-parametric test was used to compare immunoreactivities. Values for $p \leq 0.05$ were considered significant.

Table 1. MAP values for the experimental groups

Time	Group 1 (Control)	Group 2 (Dexa)	Group 3 (PQ)	Group 4 (Dexa + PQ)
0 min	121.2 ± 9.3	129.3 ± 3.1	134.33 ± 5.2[a]	138.9 ± 4.6[b]
10 min	108.0 ± 15.7[c]	135 ± 3.6[c,d]	113.5 ± 5.5[d]	115.7 ± 4.6[b]
20 min	104.3 ± 7.9[c]	131.7 ± 3.4[c]	112.4 ± 5.1[a]	111.8 ± 4.4[b]
1 h	105.1 ± 12.6	120.5 ± 3.1	100.6 ± 2.5[a]	121.6 ± 7.9
2 h	111.3 ± 12.4	130.9 ± 5.3[d]	107.7 ± 3.6[a,d]	115.3 ± 7.4[b]

Data are means ± S.E. (n = 7).
[a]PQ 0 min vs. PQ 20 min, PQ 0 min vs. PQ 1 h, PQ 0 min vs. PQ 2 h.
[b]Dexa + PQ 0 min vs. Dexa + PQ 10 min, Dexa + PQ 0 min vs. Dexa + PQ 20 min, Dexa + PQ 0 min vs. Dexa + PQ 2 h.
[c]Control 10 min vs. Dexa 10 min, Control 20 min vs. Dexa 20 min.
[d]Dexa 10 min vs. PQ 10 min, Dexa 2 h vs. PQ 2 h.
[a,b,c,d]$p < 0.05$

Results

Hemodynamic parameters

MAP and HR were recorded for all animals at 10 and 20 min, and at 1 and 2 h during the experimental period. (Tables 1, 2).

MAP for group 1 was 108.0 ± 15.7 mm Hg at 10 min and 111.3 ± 12.4 mm Hg at 2 h. (Fig.1, Table 1). HR decreased from 322.8 ± 24 to 300.0 ± 24.3/min^{-1} (Fig. 2, Table 2) for the same time interval. MAP values were decreased significantly compared to the Dexa group, especially at 10 and 20 min. Changes in HR between the control and Dexa groups were statistically significant, especially at 2 h.

MAP for group 2 was 135.0 ± 3.6 mm Hg at 10 min and 130.9 ± 5.3 mm Hg at 2 h. (Fig.1, Table 1). HR was 369 ± 15/min at 10 min and 357 ± 9/min at 2 h (Fig. 2, Table 2). MAP for group 3 decreased from 113.5 ± 5.5 mm Hg at 10 min to 107.7 ± 3.6 mm Hg at 2 h; these changes were statistically significant compared to group 2 (Fig. 1). HR for group 2 was 366 ± 7.7/min at 10 min and 366 ± 6.0/min at 2 h (Fig. 2). MAP for group 4 was 115.7 ± 4.6 mm Hg at 10 min and 115.3 ± 7.4 mm Hg at 2 h (Fig. 1). HR values increased from 357 ± 17.2/min to 378 ± 22.2/min between 10 min and 2 h in group 4; this change was not observed in the other groups (Fig. 2).

MAP was increased significantly at 10 (135.0 ± 3.6 mm Hg) and 20 min (131.7 ± 3.4 mm Hg) in group 2 compared to the other groups (Fig. 1, Table 1). Also, compared to group 2 (120.5 ± 3.1 mm Hg) and group 4 (121.6 ± 7.9 mm Hg), MAP was decreased significantly ($p < 0.05$) at 1 h in group 3 (100.6 ± 2.5 mm Hg). HR also was increased significantly in groups 2 − 4 compared to group 1 at 2 h.

Immunohistochemistry

Micrographs of the immunohistochemical staining of the groups are shown in Fig. 3; the staining intensities and statistical analyses of the groups are given in Table 3. In kidney sections from the control group, COX-1 and COX-2 immunoreactivities were moderate (3.75 ± 0.50) in the glomeruli, loops of Henle, vasa

Table 2. HR values for the experimental groups

	Group 1 (Control)	Group 2 (Dexa)	Group 3 (PQ)	Group 4 (Dexa + PQ)
0 min	312.0 ± 22.7	357 ± 21.6	378 ± 3.5	366 ± 7.7
10 min	322.8 ± 24.0	369 ± 15.0	366 ± 7.7	357 ± 17.2
20 min	314.4 ± 26.4[a]	339 ± 35.5	372 ± 4.9[a]	363 ± 20.4
1 h	312.0 ± 4.3[a]	351 ± 19.8	363 ± 13.3[a]	360 ± 21.4
2 h	300.0 ± 24.3[a,b,c]	357 ± 9.0[b]	366 ± 6.0[a]	378 ± 22.2[c]

Data are means ± S.E. (n = 7).
[a]Control 20 min vs. PQ 20 min, Control 1 h vs. PQ 1 h, Control 2 h vs. PQ 2 h.
[b]Control 2 h vs. Dexa 2 h.
[c]Control 2 h vs. Dexa + PQ 2 h.
[a,b,c]$p < 0.05$.

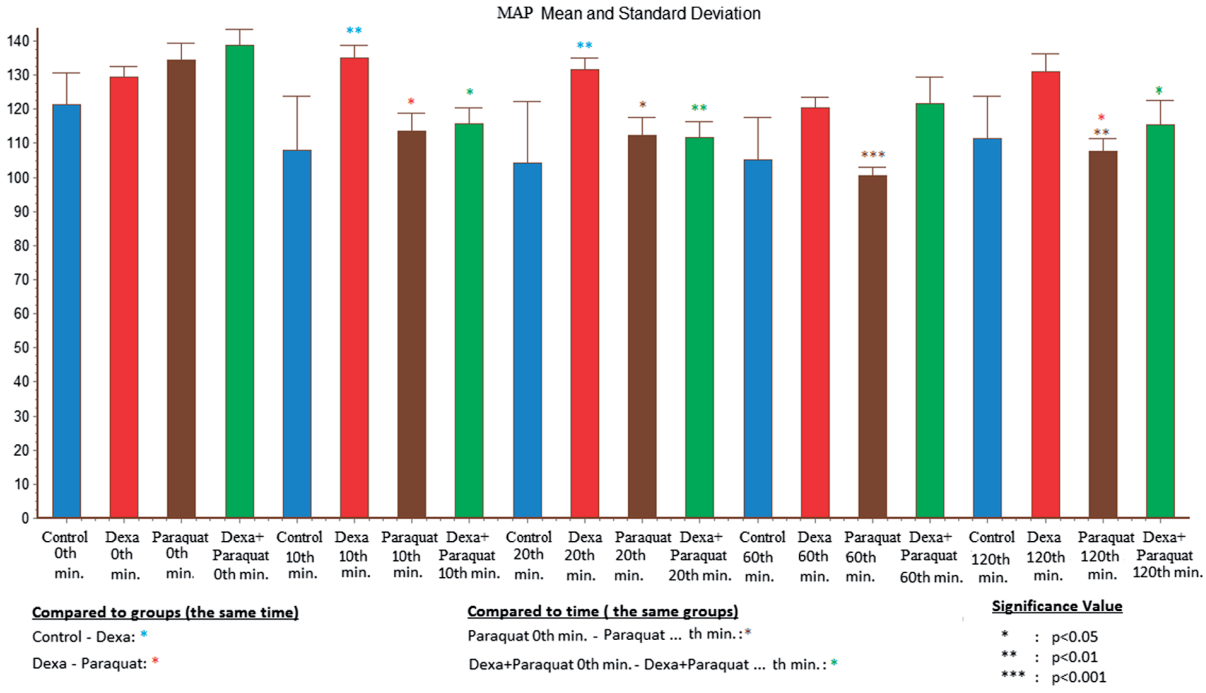


Fig. 1. MAP graphics of the experimental groups.

recta and capillaries, whereas no immunoreactivity was observed in the distal and collecting tubules (Fig. 3). We observed that ACE immunoreactivity was more intense (4.50 ± 0.57) than COX-1 and COX–2 immunoreactivities in the same locations. AQU-1 and VCAM immunoreactivities were very strong in the blood vessels, vasa recta and apical regions of the proximal tubules. (Table 3, Fig. 3A, E, I, M, R).

In sections from group 2, immunoreactivities of COX-1, COX-2 and ACE were minimal (0.75 ± 0.50) and AQU-1 immunoreactivity was weak to mild (2.75 ± 0.95) in the renal cortex. VCAM

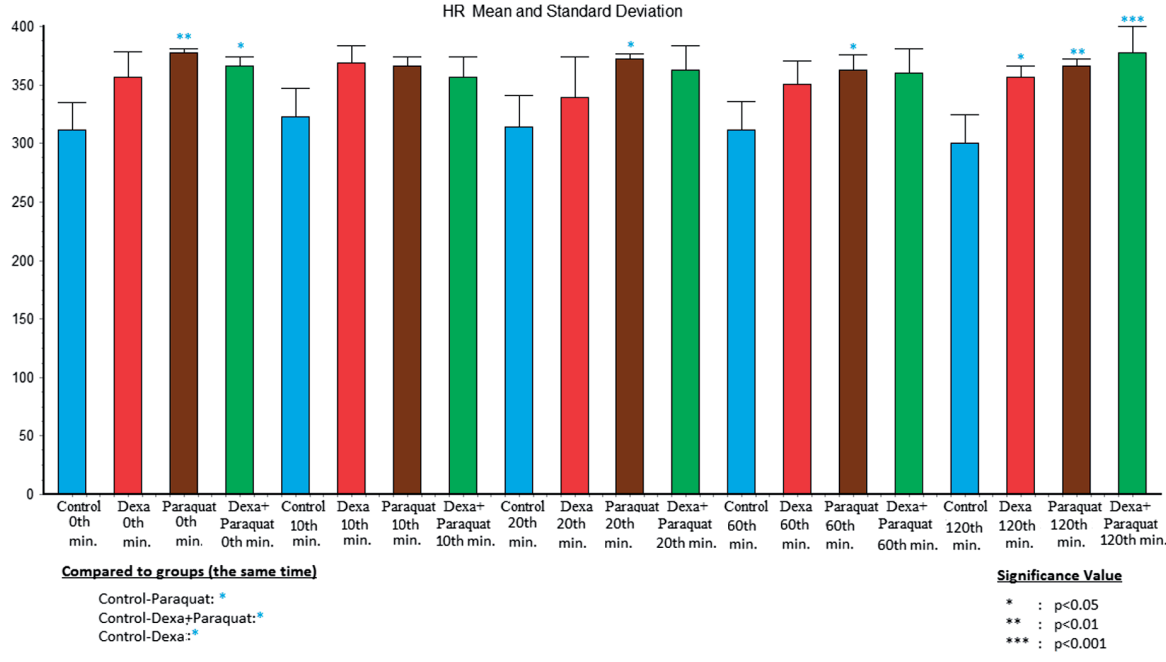


Fig. 2. HR graphics of the experimental groups.

immunoreactivity was moderate (4.25 ± 0.50) in group 2. Immunoreactivities decreased significantly in group 2 compared to groups 1, 3 and 4 (Fig. 3B, F, J, N, S).

In sections from group 3, we observed widened Bowman spaces and vacuolization in the proximal renal tubules. Immunoreactivities of COX-1 and COX-2 were minimal to weak (1.25 ± 0.50). ACE immunoreactivity was weak (1.50 ± 0.57), AQU-1 was very strong (5.75 ± 0.50) and VCAM was very strong (5.50 ± 0.57) in the renal cortex (Fig. 3C, G, K, O, T).

In sections from group 4, vacuolizations were fewer than in group 3 in the proximal tubules. COX-1 and COX-2 immunoreactivities were moderate (3.75 ± 0.50). ACE immunoreactivity was weak (2.75 ± 0.50) in group 4. AQU-1 immunoreactivity was similar to group 1 (controls) in the proximal tubules and vessels (5.50 ± 0.57). VCAM immunoreactivity was moderate to strong (4.50 ± 0.57) in the renal cortex and capillaries. (Fig. 3D, H, L, P, U).

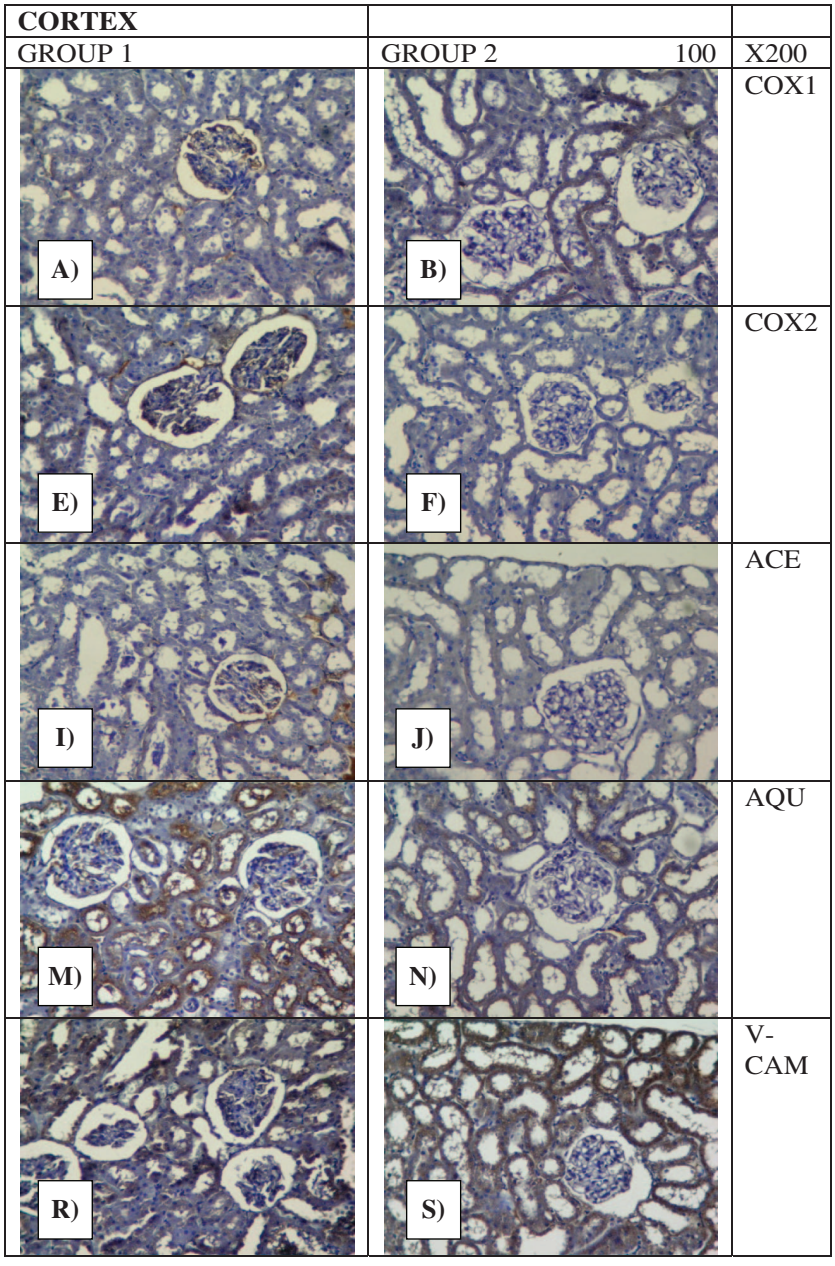


Fig. 3. Kidney cortex samples collected from the experiment groups. All immunoreactivities were decreased compared to controls and the immunoreactivities that markedly decreased in group 2 approached normal in group 4. A–D) COX-1. E–H) COX-2. I–L) ACE. M–P) AQU-1. R–V) VCAM. × 400.

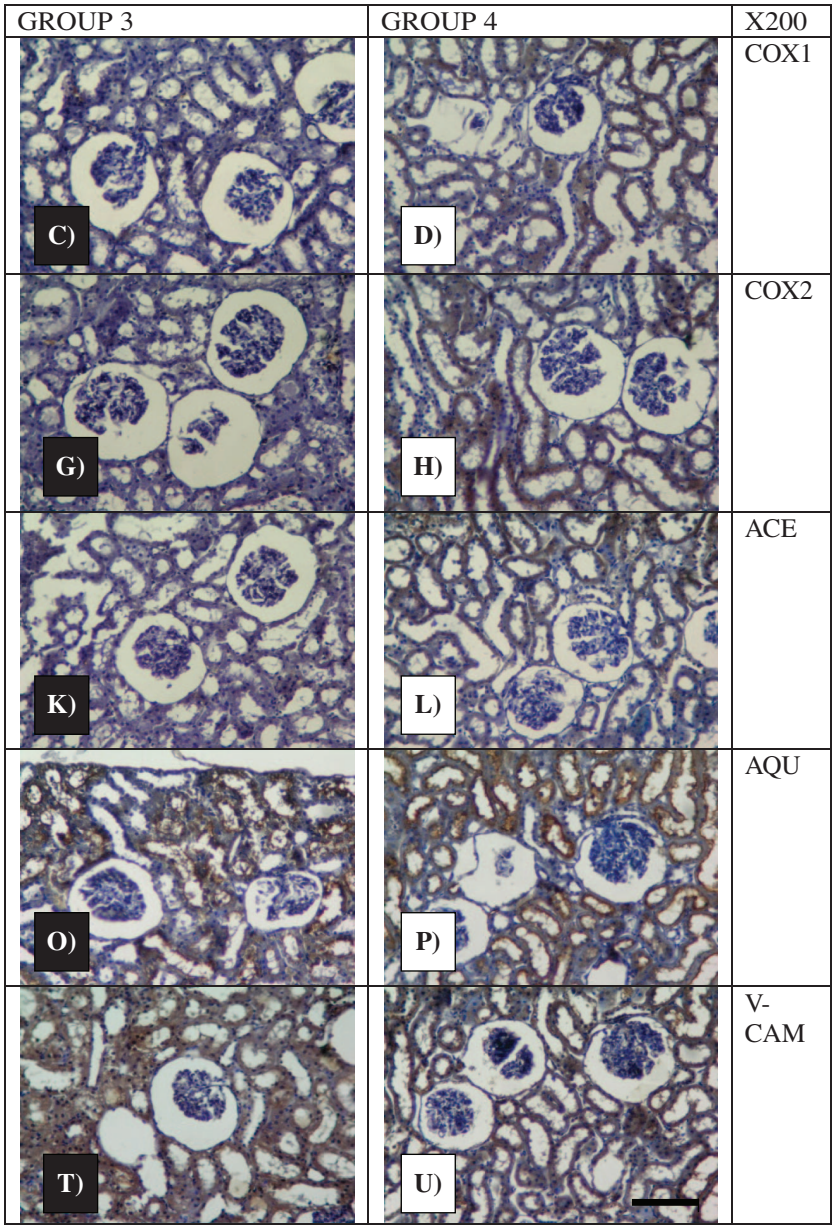


Fig. 3. *Continued*.

Discussion

PQ is a common herbicide that in humans causes pulmonary fibrosis and nephrotoxicity. We investigated the hemodynamic changes in rat kidney caused by PQ poisoning using histochemical and immunohistochemical methods.

Glucocorticoids play an important role in control of blood pressure and vascular tonus. Glucocorticoid treatment for extended periods causes high blood pressure (Clifton et al. 2002). Goodwin et al. (2008) suggested that the glucocorticoid receptor in vascular smooth muscle plays a critical role in the acute hypertensive response to glucocorticoids in vivo. It has been suggested that glucocorticoids cause high blood pressure by inhibiting cyclooxygenase and nitric oxide synthase stimulated synthesis of endotelin-1, dihydropiridin-sensitive type L calcium channels and by increasing intracellular concentration of calcium (Clifton et al. 2002). Although it is believed that glucocorticoids increase blood pressure by activating mineralocorticoid receptors in the kidneys, it appears that there is more than one mechanism (Goodwin et al. 2008); glucocorticoids also affect smooth muscle cells of vessels and vessel endothelium. It has been shown

Table 3. Intensities of immunoreactivities of COX-1, COX-2, ACE, AQU-1 and VCAM in rat kidney cortex

	Group 1 (Control)	Group 2 (Dexa)	Group 3 (PQ)	Group 4 (PQ + Dexa)
COX-1	$3.75 \pm 0.50^{a,b}$	$0.75 \pm 0.50^{a,c}$	$1.25 \pm 0.50^{b,d}$	$3.75 \pm 0.50^{c,d}$
COX-2	$3.75 \pm 0.50^{a,b}$	$0.75 \pm 0.50^{a,c}$	$1.25 \pm 0.50^{b,d}$	$3.75 \pm 0.50^{c,d}$
ACE	$4.50 \pm 0.57^{a,b}$	$0.75 \pm 0.50^{a,c}$	1.50 ± 0.57^{b}	2.75 ± 0.50^{c}
AQU-1	5.75 ± 0.50^{a}	$2.75 \pm 0.95^{a,c,f}$	5.75 ± 0.50^{f}	5.50 ± 0.57^{c}
VCAM	$5.75 \pm 0.50^{a,e}$	$4.25 \pm 0.50^{a,f}$	5.50 ± 0.57^{f}	4.50 ± 0.57^{e}

Data are means ± S.E. (n = 7).
[a]Control COX1 vs. Dexa COX1, Control COX2 vs. Dexa COX2, Control ACE vs. Dexa ACE, Control AQU1 vs. Dexa AQU1, Control VCAM vs. Dexa VCAM.
[b]Control COX1 vs. PQ COX1, Control COX2 vs. PQ COX2, Control ACE vs. PQ ACE.
[c]Dexa COX1 vs. PQ + Dexa COX1, Dexa COX2 − PQ + Dexa COX2, Dexa ACE − PQ + Dexa ACE, Dexa AQU1 vs. PQ + Dexa AQU1.
[d]PQ COX1 vs. PQ + Dexa COX1, PQ COX2 vs. PQ + Dexa COX2.
[e]Control VCAM vs. PQ + Dexa VCAM.
[f]Dexa AQU1 vs. PQ AQU1, Dexa VCAM vs. PQ VCAM.
[a,b,c,d,e,f]$p < 0.05$.

that glucocorticoids increase angiotensin 1 receptors on smooth muscle cells and alter the intake of Na^+ and/or Ca^{2+} (Goodwin et al. 2008).

Based on experimental models, glucocorticoids up-regulate angiotensin II type 1 receptors, and α-1 receptors in vascular smooth muscle may augment adipocyte production of angiotensinogen and stimulate angiotensin converting enzyme expression while reducing prostacyclin E2 synthesis by the endothelium and vascular smooth muscle. Brotman et al. (2005) reported decreased HR after Dexa administration, which suggests that increased central sympathetic tone did not contribute to the increase in blood pressure**.** These investigators also reported that they found no evidence that Dexa diminished endothelium-mediated vasodilatation.

MAP was increased more in the group treated with Dexa at high dose at 10 and 20 min, and at 2 h than in the other groups. The significant decrease in MAP in the PQ group was evident at 1 h; however, in the Dexa+ PQ group, MAP values were ameliorated by Dexa treatment. COX-1 and COX-2 can be affected by Dexa administration. Glucocorticoids cause hypertension by inhibiting cyclo-oxygenase, up-regulating dihydropyridine sensitive L-type calcium channels and by other pathways associated with increased intracellular calcium concentration (Clifton et al. 2002). Dexa, COX-2 (Goppelt-Struebe et al. 2000) and inducible nitric oxide synthase (iNOS) suppress vasodilator processes by inhibiting transcription of NF-kappaB/IkappaB alpha (Yokota et al. 2007). We found that Dexa suppressed COX-1 and COX-2 immunoreactivity significantly.

In mammalian tissues, COX-2 regulates vascular tone, and expression and secretion of renin; it also affects salt and water homeostasis in kidney. Hypertonic sodium permits activation of COX-2 in the kidney medullary interstitial cells by activating epidermal growth factor receptors. Transcription factors, such as NF-kB and mitogen-activated protein kinase (MAPK), participate in regulating COX-2 expression in a high salt environment. Three other members of the MAPK family, ERK, JNK-2, and p38, are required for expression of tonicity-induced COX-2 in the inner medullary collecting duct cells (Lima et al. 2005).

Leon et al. (2001) demonstrated the presence of COX-2 in the thick ascending limb of Henle cells in normal adult rat and its regulation by glucocorticoids. There also is evidence that COX-2 is regulated by dietary salt intake, angiotensin II, corticosteroid and furosemide. Furthermore, COX-2-derived prostaglandins participate in renin secretion, tubuloglomerular feedback, and sodium handling.

Rajashree et al. (1999) reported that Dexa caused increased MAP, significant alterations in electrocardiographic parameters, marked alterations of enzymatic and nonenzymatic antioxidant levels, and in the TBA reactants level in heart and kidney. Rajashree et al. (1999) reported that the level of ACE enzyme was increased in both serum and tissues, which may play a role in Dexa induced hypertension.

Li et al. (2008) reported that in rats given Dexa, average MAP, urine flow, sodium and urea excretion rates, and tissue AQU-1 in the kidney were increased significantly. This result differs from ours and may be related to the acute high doses used in our study. In addition, the promotor of AQP-1 contains a glucocorticoid response element. Tran et al. (2010) claimed that AQU-1 may be involved with

edema-related brain injury and might be controlled by external conditions such as the pH and the presence of steroids. We also found that high doses of Dexa may alter the expression of AQU-1.

VCAM-1 and ICAM-1 expressions are increased by immune system diseases. These adhesion molecules encourage inflammatory cells to adhere to the parenchymal target cells. Wuthrich et al. (1993) studied the effects of Dexa on adhesion molecules such as VCAM-1 and ICAM-1 under physiologic conditions. We found that VCAM immunoreactivity was moderately decreased in Dexa treated group.

Because we designed our study to evaluate the acute effects of Dexa on PQ poisoning, we could not assess signs of fibrosis. We found that PQ administration caused expansion of Bowman spaces and vacuolization in the tubule epithelium. Malekinejada et al. (2013) reported proximal tubule degeneration, increased basement membrane thickness and degenerated glomeruli in PQ exposed animals and reported that PQ up-regulated COX-2 expression in the kidney tissue. Our findings confirmed that PQ caused marked alterations of kidney morphology and that these were attenuated by Dexa treatment.

Dexa causes hemodynamic changes even during the acute phase of PQ intoxication. AQU-1, COX-1, COX-2 and ACE immunoreactivities were reduced significantly in the Dexa treated group, whereas in PQ + Dexa group, immunoreactivities were close to those of the control group. After acute PQ intoxication, Dexa may prevent cyclo-oxygenase release by suppressing inflammation and edema in renal tissue. Dexa treatment also suppressed VCAM and AQU-1.

We investigated the hemodynamic effects of Dexa treatment on acute PQ intoxication in rat kidney. Immunohistochemical staining of COX-1 and -2, ACE, AQU-1 and V-CAM was consistent with the ameliorative effects of Dexa on PQ toxicity. Therefore, it may possible to use Dexa to treat PQ intoxication.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Benga G (2012) The first discovered water channel protein, later called aquaporin. 1. Molecular characteristics, functions and medical implications. *Mol. Asp. Med.* 33: 518–534.

Bismuth C, Garnier R, Baud FJ, Muszynski J, Keyes C (1990) Paraquat poisoning. An overview of the current status. *Drug Safety* 5: 243–251.

Brotman DJ, Girod JP, Garcia MJ, Patel JV, Gupta M, Posch A, Saunders S, Lip GY, Worley S, Reddy S (2005) Effects of short-term glucocorticoids on cardiovascular biomarkers. *J. Clin. Endocrinol. Metab.* 90: 3202–3208.

Carlos TM, Harlan JM (1994) Leukocyte endothelial adhesion molecules. *Blood* 84: 2068–2101.

Chen HW, Tseng TK, Ding LW (2009) Intravenous paraquat poisoning. *J. Chin. Med. Assoc.* 72: 547–550.

Clifton VL, Wallace EM, Smith R (2002) Short-term effects of glucocorticoids in the human fetal-placental circulation in vitro. *J. Clin. Endocrinol. Metab*. 87: 2838–3282.

Dinis-Oliveira RJ, Duarte JA, Remiao F, Sanchez-Navarro A, Bastos ML, Carvalho F (2006) Single high dose dexamethasone treatment decreases the pathological score and increases the survival rate of paraquat-intoxicated rats. *Toxicology* 227: 73–85.

Dinis-Oliveira RJ, Duarte JA, Sánchez-Navarro A, Remião F, Bastos ML, Carvalho F (2008) Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment. *Crit. Rev. Toxicol*. 38: 13–71.

Dinis-Oliveira RJ, Pontes H, Bastos ML, Remião F, Duarte JA, Carvalho F (2009) An effective antidote for paraquat poisonings; the treatment with lysine acetylsalicylate. *Toxicology* 255: 187–193.

Goodwin JE, Zhang J, Geller DS (2008) A critical role for vascular smooth muscle in acute glucocorticoid-induced hypertension. *J. Am. Soc. Nephrol*. 19: 1291–1299.

Goppelt-Struebe M, Rehm M, Schaefers HJ (2000) Induction of cyclo-oxygenase-2 by platelet-derived growth factor (PDGF) and its inhibition by dexamethasone are independent of NF-kB/IkB transcription factors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 361: 636–645.

Hench PS (1949) The effect of a hormone of the adrenal cortex (17-hydroxy-11-dehydrocorticosterone: compound E) and of pituitary adrenocorticotropic hormone on rheumatoid arthritis: preliminary report. *Ann. Rheum*. 8: 97–104.

Herrera EA, Verkerk MM, Derks JB, Giussani DA (2010) Antioxidant treatment alters peripheral vascular dysfunction induced by postnatal glucocorticoid therapy in rats. *PLoS One* 5: 9250.

Imai Y, Kuba K, Penninger JM (2007) Angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Cell Mol. Life Sci*. 64: 2006–2012.

Leon CG, Marchetti J, Vio CP (2001) Renal cyclooxygenase-2: evidence for recruitment of thick ascending limb of Henle cells in microdissected nephron segments. *Hypertension* 38: 630–634.

Li C, Wang W, Summer SN, Falk S, Schrier RW (2008) Downregulation of UT-A1/UT-A3 is associated with urinary concentrating defect in glucocorticoid-excess state. *J. Am. Soc. Nephrol*. 19: 1975–1981.

Lima WC, Parka M, Bahna JJ, Inoueb H, Lee YJ (2005) Hypertonic sodium chloride induction of cyclooxygenase–2 occurs independently of NF-kB and is inhibited by the glucocorticoid receptor in A549 cells. *FEBS Lett.* 579: 5430–5436.

Lin JL, Lin-Tan DT, Chen KH, Huang WH (2006) Repeated pulse of methylprednisolone and cyclophosphamide with continuous dexamethasone therapy for patients with severe paraquat poisoning. *Crit. Care Med.* 34: 368–373.

Malekinejada H, Rezabakhsha A, Rahmanib F, Razic M (2013) Paraquat exposure up-regulates cyclo-oxygenase-2 in the lungs, liver and kidneys in rats. *Iran J. Pharmaceut. Res*. 12: 887–896.

Meng JP, Ceryak S, Aratsu Z, Jones L, Epstein L, Bouscarel B (2006) Biphasic regulation by bile acids of dermal fibroblast proliferation through regulation of cAMP production and COX-2 expression level. *Am. J. Physiol. Cell Physiol.* 291: 546–554.

Neeck G (2002) Fifty years of experience with cortisone therapy in the study and treatment of rheumatoid arthritis. *Ann. NY Acad. Sci.* 966: 28–38.

Onyeama HP, Oehme FW (1984) A literature review of paraquat toxicity. *Vet. Hum. Toxicol.* 26: 494–502.

Rajashree S, Puvanakrishnan R (1999) Dexamethasone induced alterations in enzymatic and nonenzymatic antioxidant status in heart and kidney of rats. *Mol. Cell Biochem.* 197: 203–208.

Samai M, Sharpe MA, Gard PR, Chatterjee PK (2007) Comparison of the effects of the superoxide dismutase mimetics EUK-134 and tempol on paraquat-induced nephrotoxicity. *Free Rad. Biol. Med*. 43: 528–534.

Tran ND, Kim S, Vincent HK, Rodriguez A, Hinton DR, Bullock MR, Young HF (2010) Aquaporin-1-mediated cerebral edema following traumatic brain injury: effects of acidosis and corticosteroid administration. *J. Neurosurg*. 112: 1095–1104.

Wuthrich RP, Sekar P (1993) Effect of dexamethasone, 6-mercaptopurine and cyclosporine A on intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 expression. *Biochem. Pharmacol*. 19: 1349–1353.

Yokota A, Taniguchi M, Takahira Y, Tanaka A, Takeuchi K (2007) Dexamethasone damages the rat stomach but not small intestine during inhibition of COX-1. *Dig. Dis. Sci*. 52: 1452–1461.

Zeidel ML, Ambudkar SV, Smith BL, Agre P (1992) Reconstitution of functional water channel in liposomes containing purified red cell CHIP28 protein. *Biochemistry* 31: 7436–7440.