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Effects of captopril on the development of rat doxorubicin nephropathy

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Summary. The effects of a daily administration of an anti-converting enzyme inhibitor, Captopril (CPT) (100 mg/kg/orally), on the development of functional and morphological alterations induced in rats by a single injection (7.5 mg/kg/iv) of Doxorubicin (DXR) (Adriamycin*), were investigated. Twenty-four-hour protein excretion, urine output, food intake, water intake, and body weight gain were measured weekly for 30 days. Transmission and scanning electron microscopy observations were performed on kidney samples after 30 days. Four groups were studied. Group 1 were control rats. Group 2 were rats injected with DXR. Group 3 were rats injected with DXR and treated with CPT for 30 days. Group 4 were rats injected with DXR and treated with CPT for 15 days (CPT treatment started 15 days after DXR injection).

Group 1 did not show significant functional or morphological changes. Group 2 showed severe proteinuria, significant increase in urinary volume within 2 weeks, significant body weight reduction and diffuse morphological changes. These changes mainly consisted of podocyte swelling, severe foot process fusion, and presence of casts within tubular lumen. Group 3, with respect to group 2, showed a significant reduction of the 24 h protein excretion and urine output. This group displayed morphological changes similar to those observed in group 2, but with a focal distribution. Group 4 showed functional and morphological changes comparable with those of group 2.

It is concluded that CPT partially inhibits the development of the functional and morphological damage induced by DXR in the rat kidney. However, CPT did not influence the natural development of nephropathy when treatment started 15 days after DXR injection.

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Introduction

Intrarenal renin-angiotensin system (RAS) is involved in the regulation of glomerular hemodynamics (Brener et al., 1982). Angiotensin II (A II) increases efferent arteriolar resistance and/or preglomerular resistance (Rosivall and Navar, 1983). As a result of a greater efferent than afferent arteriolar effect of A II, glomerular capillary hydrostatic pressure and capillary pressure gradient increases. These events reduce the effective filtration area (Blantz and Gabbai, 1987). Experimental evidences also suggest that RAS may induce and/or even maintain renal hemodynamic abnormalities that lead to progressive glomerular injury and to renal insufficiency (Meggs and Hollenberg, 1980). Numerous studies have demonstrated that angiotensin converting enzyme (ACE) inhibitors slow down the development of glomerular lesions in the remnant kidney of subtotally nephrectomized rats (Anderson et al., 1985; Meyer et al., 1985).

This study was performed in order to evaluate the effects of Captopril (CPT) (100 mg/kg/daily/orally), an ACE inhibitor, on the progression of renal functional and ultrastructural changes induced by Doxorubicin (DXR) (Adriamycine*) injection in rats. DXR nephropathy in rats was used as a tool to investigate experimentally progressive renal failure. The administration of DXR, an anthracycline antibiotic (Grond et al., 1984) to rats, induces the development of a nephrotic syndrome that mimics minimal change nephropathy in humans (Raij and Michael, 1980). It was shown that CPT does not protect from DXR-induced nephropathy (Hall et al., 1986; Beukers et al., 1988) or, surprisingly, that it may worsen the renal function (Hall et al., 1986). In the present paper,

functional and morphological evidences of a CPT protective effect on the DXR-induced nephropathy in the rat are reported.

Materials and methods

Experimental protocol

Thirty-two adult, male Wistar rats, (weight 200-250 g), were housed in a room with constant temperature and humidity, and a photoperiod of 12 hr on, 12 hr off. The animals had free access to standard laboratory rat chow and tap water. Doxorubicine (DXR) nephropathy was induced by a single intravenous injection (iv.i.) of DXR at a dose of 7.5 mg/kg. Treatment was performed with Captopril (CPT) (100 mg/kg/die) administered by oral gavage. Four groups were studied. Group 1 (control) after 2 days from a single dose of a saline solution iv.i., underwent a daily oral administration of a saline solution for 30 days. Group 2, after 2 days from DXR iv.i., underwent a daily oral administration of a saline solution for 30 days. Group 3, after two days from DXR iv.i., underwent CPT treatment for 30 days. Group 4, after 15 days from DXR iv.i., underwent CPT treatment for 15 days.

Animals were placed in metabolic cages for 24 hour urine collection, performed 2 days prior to (time 0), and 1, 2, 3 and 4 weeks after, DXR iv.i. Twelve hours acclimatisation to the cages were allowed before starting 24 hr collection period. Water and food intake, body weight gain and urine output were recorded. Proteinuria (mg/24 h) was estimated with sulphosalicylic acid method. After treatment rats were sacrificed and electron microscopy observations were performed on the kidney cortex.

Drugs

DXR, (Adriamycin, Adriblastina) was obtained from Farmitalia Carlo Erba. CPT was kindly provided by Squibb Italia Spa. DXR was dissolved in H_2O and CPT in 0.15 M NaCl solution. Control animals received an equivalent volume of vehicle.

Sampling procedure and electron microscopy

After ether anesthesia rats underwent laparotomy, exposure of the abdominal aorta and inferior vena cava. Right kidney vessels were campled, right nephrectomy was performed and right kidney weight was recorded. Left common iliac artery was catheterised with a catheter (PE-50) which was positioned in the abdominal aorta just below the left renal artery branch. Saline washing solution and 2.5% glutaraldehyde in phosphate buffer solution were injected at physiological pressure (Fujita and Miyoshi, 1984). Then, the left kidney was removed, sectioned and stored for 12 hours in the same refrigerated fixative. For transmission electron microscopy (TEM) observations, fragments of the cortical region of the samples were postfixed in osmium tetroxide, dehydrated in alcohol substituted with propylene oxide, and embedded in Epon. Sections were cut with an LKB Ultratome III. Semithin sections were stained with metylene blue. Ultrathin sections were contrasted with lead citrate and uranyl acetate and observed in a Zeiss EM9 SA. For scanning electron microscopy (SEM) observations, the samples were post-fixed with osmium tetroxide, dehydrated in alcohol, critical-point dryed with CO_2, glued by means of a silver paint on aluminium stubs, sputtered with gold and observed in a Cambridge Stereoscan 150 SEM. All the samples were maintained without identification during observation. A minimum of 50 glomeruli were studied (25 by means of SEM, and 25 by means of TEM), amounting to a total of 200 glomeruli for each experimental group.

Statistical evaluation of the functional data

Data are expressed as mean ± S.D. Comparison between means of two groups was performed using analysis of variance (ANOVA) and considered significant at the $p < 0.05$ level.

Results

Functional data

Body weight

In group 2, DXR injection significantly reduced body weight gain compared to the control group 1. As shown in Table 1 the CPT treatment regimens did not influence the body weight gain neither in group 3 nor in group 4 (Table 1).

Food intake

Food intake decreased in group 2 compared to group 1. Similar changes were seen in groups 3 and 4 (Table 2).

Water intake

In Group 1 water intake physiologically tended to decrease relative to the increase in body weight. Group 2 did not show this physiological reduction in water intake. Similarly, neither group 3 nor group 4 showed improvement of this impaired physiological pattern (Table 3).

Urinary output

Group 2 had a marked increase in urinary volume (Table 4) compared to group 1. In group 3 the urinary volume was significantly reduced compared to group 2 during the first two weeks of treatment, but was comparable to those observed in group 2 during the

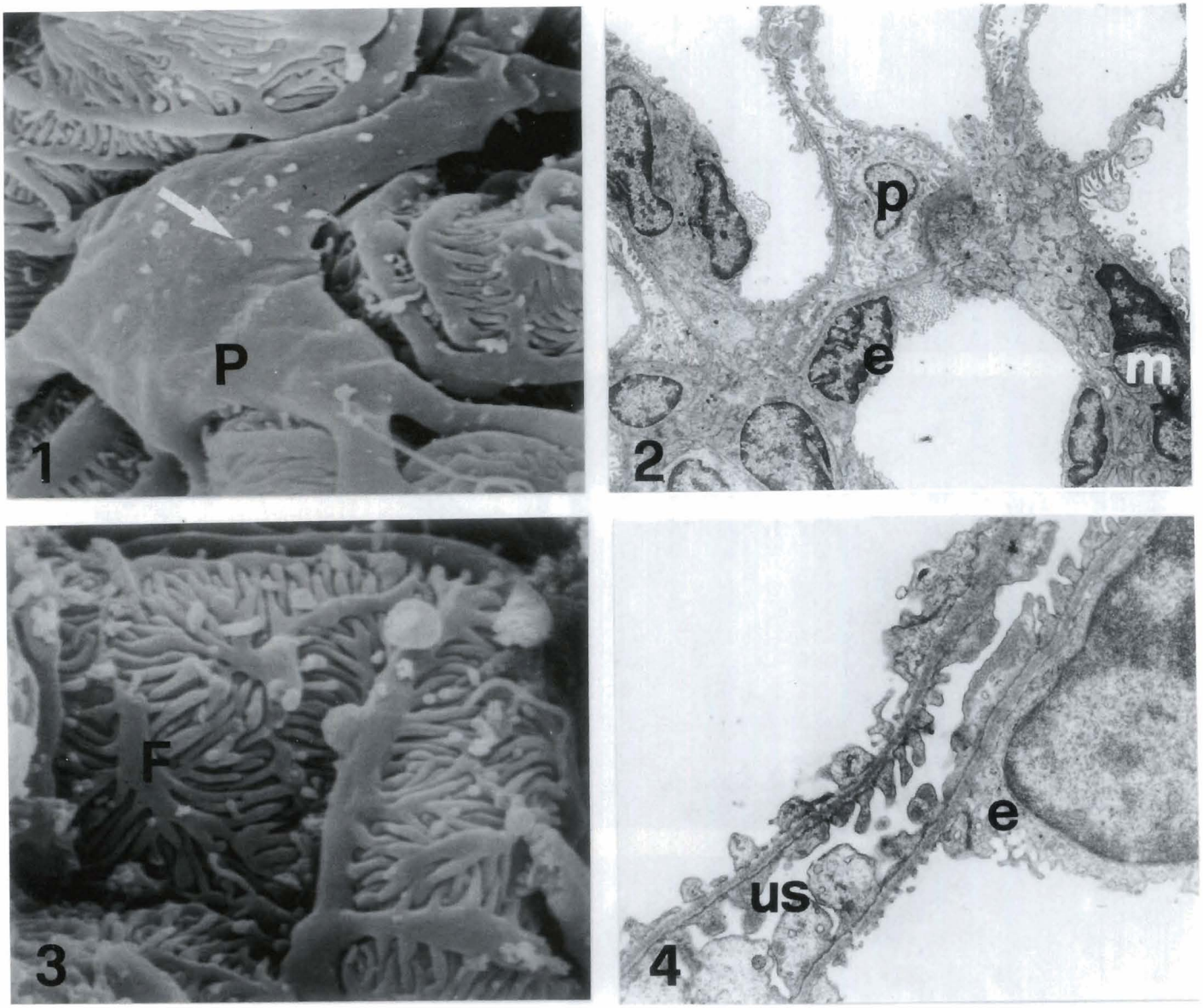


Fig. 1. SEM of Group 1 sample. Cell body of a podocyte (P) with small surface projections (arrow) × 15,000

Fig. 2. TEM of Group 1 sample. Podocyte (p), endothelial cells (e) and mesangial cells (m) are well preserved. × 5,000

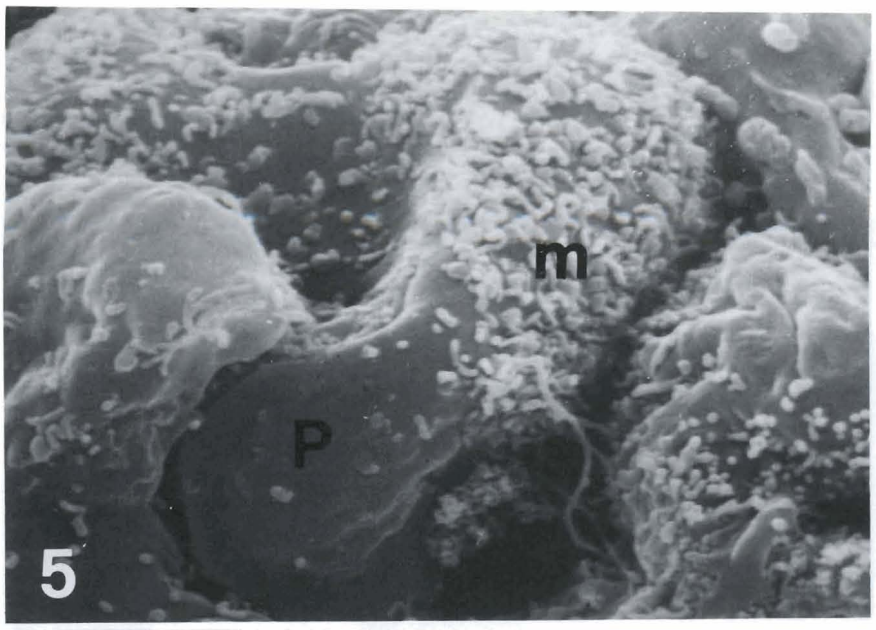
Fig. 3. SEM of Group 1 sample. Note the fine three-dimensional arrangement of podocyte foot processes (F). × 18,000

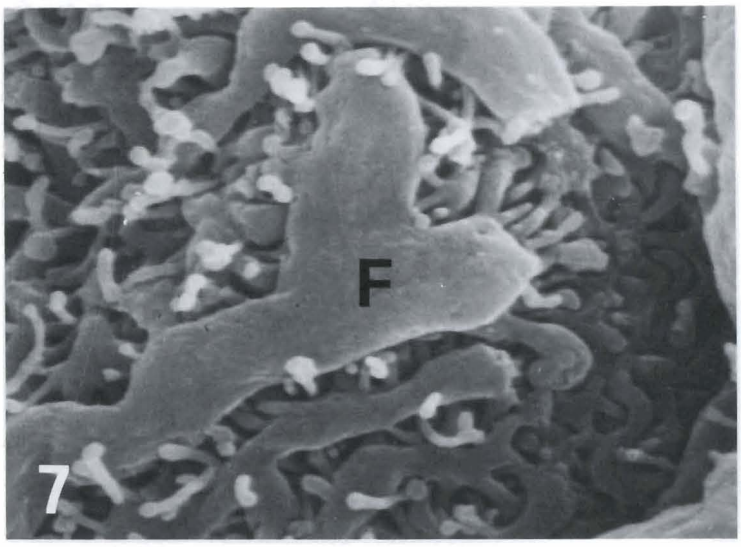
Fig. 4. TEM of Group 1 sample. Podocyte foot processes show normal ultrastructural appearance. (us = urinary space; e = endothelial cell). × 18,000

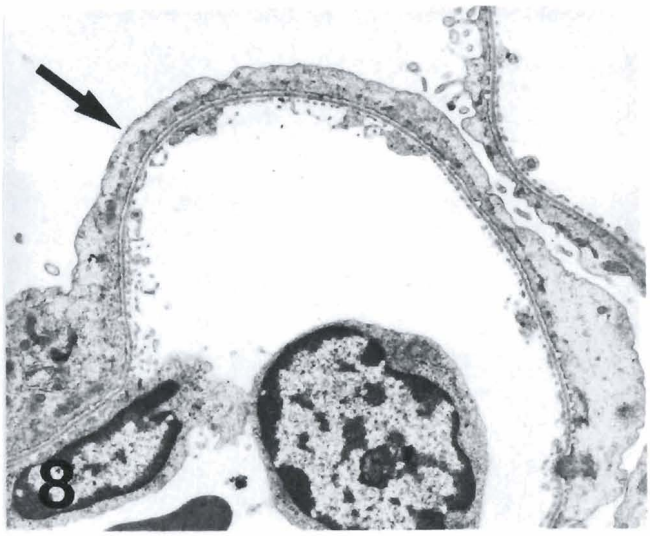
Table 1. Effects of Captopril (CPT; 100 mg/kg/os/day) on body weight gain (g) in rats with doxorubicin (DXR) nephropathy.

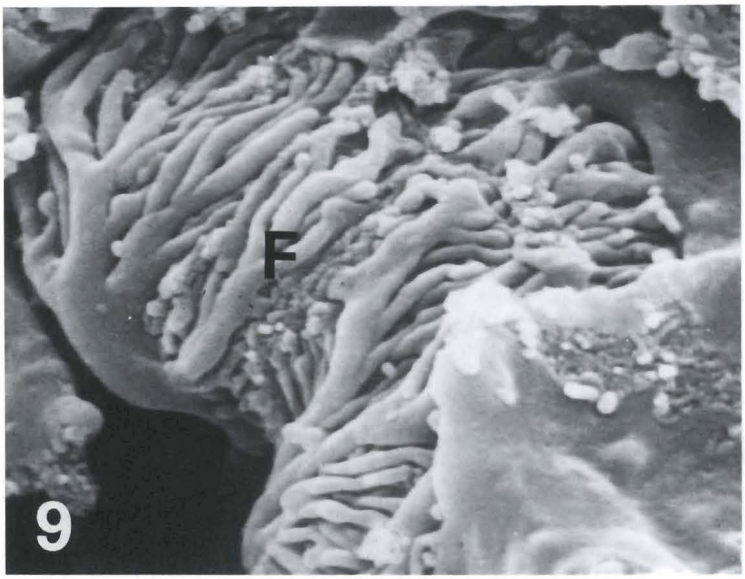
	Weeks of treatment				
	0	1	2	3	4
Group 1	228 ± 14	236 ± 15	301 ± 22	347 ± 23	382 ± 20
Group 2	227 ± 18	237 ± 18	245 ± 17*	249 ± 15*	255 ± 18*
Group 3	230 ± 20	231 ± 19	267 ± 15*	227 ± 18*	262 ± 15*
Group 4	225 ± 16	235 ± 13	244 ± 20*	251 ± 18*	256 ± 16*

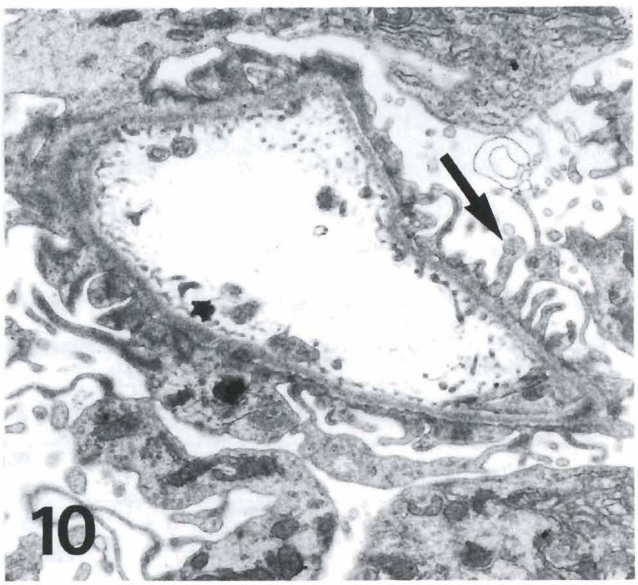
Group 1 = Control; Group 2 = DXR untreated; Group 3 = DXR + CPT for 4 weeks; Group 4 = DXR + CPT for 2 weeks
Data are expressed as mean ± S.D. Data were analyzed using the analysis of variance.
*$p < .05$ versus CTRL.











Figs. 5-6. SEM and TEM of Group 2 sample. Podocyte cell body (P) shows numerous blebs and microvilli (m) proliferating within the urinary space (us). × 15,000

Figs. 7-8. SEM and TEM of Group 2 sample. Foot processes (F) disorganization and fusion are clearly seen. The foot processes lose their shape and are replaced by epithelial cytoplasmic projections (arrow). × 20,000 and × 9,000

Figs. 9-10. SEM and TEM of Group 3 sample. Podocyte cytoplasmic processes (F) are swollen and show effacement. However, their three-dimensional organization as well as their shape (arrow) show a better preservation than seen in Doxorubicin injected samples (Group 2). × 18,000 and × 9,000

Table 2. Effects of Captopril (CPT; 100 mg/kg/os/day) on food intake (g) in rats with doxorubicin (DXR) nephropathy.

	Weeks of treatment				
	0	1	2	3	4
Group 1	24 ± 4	27 ± 6	33 ± 6	35 ± 6	35 ± 7
Group 2	26 ± 5	22 ± 2	21 ± 4*	20 ± 3*	21 ± 3*
Group 3	25 ± 5	23 ± 4	23 ± 3*	22 ± 4*	21 ± 2*
Group 4	22 ± 3	24 ± 2	25 ± 3*	21 ± 4*	20 ± 5*

Data are expressed as mean ± S.D. Data were analyzed using the analysis of variance.
*P < .01 versus group 1

Table 3. Effects of Captopril (CPT; 100 mg/kg/os/day) on water intake (ml/100 g body weight) in rats with doxorubicin (DXR) nephropathy.

	Weeks of treatment				
	0	1	2	3	4
Group 1	15 ± 2	13 ± 2	11 ± 4	8 ± 1	8 ± 3
Group 2	14 ± 1	12 ± 3	12 ± 2	12 ± 3*	15 ± 2*
Group 3	15 ± 3	12 ± 4	11 ± 2	13 ± 2*	17 ± 2*
Group 4	14 ± 4	13 ± 2	13 ± 4	13 ± 2*	16 ± 3*

Data are expressed as mean ± S.D. Data were analyzed using the analysis of variance.
*P < .001 group 1

Table 4. Effects of Captopril (CPT; 100 mg/kg/os/day) on urinary volume (ml/24 h) in rats with doxorubucin (DXR) nephropathy.

	Weeks of treatment				
	0	1	2	3	4
Group 1	5 + 1	6 + 2	6 ± 0.2	6 + 0.7	6 ± 0.9
Group 2	6 ± 1	5 ± 0.8	8 ± 0.2*	10 ± 1*	10 ± 0.9*
Group 3	5 ± 0.5	6 ± 1	6 ± 0.2*	7 ± 0.4*	11 ± 0.5*
Group 4	7 ± 2	7 ± 0.8	9 ± 2*	11 ± 0.1*	12 ± 0.6*

Data are expressed as mean ± S.D. Data were analyzed using the analysis of variance.
*P < .01 group 1
*P < .05 group 2

Table 5. Effects of Captopril (CPT; 100 mg/kg/os/day) on urinary protein excretion (mg/24 h) in rats with (DXR) doxorubicin nephropathy.

	Weeks of treatment				
	0	1	2	3	4
Group 1	6.2 ± 1.1	5.9 ± 1.6	6.7 ± 1.7	6.5 ± 1.1	6.8 ± 1.3
Group 2	7.1 ± 1.5	70 ± 7	272 ± 13*	425 ± 43*	478 ± 36*
Group 3	6.5 ± 0.9	20 ± 4*	176 ± 11*	260 ± 27*	270 ± 26*
Group 4	7.5 ± 2.1	75 ± 5	290 ± 19*	420 ± 31*	412 ± 25*

Data are expressed as mean ± S.D. Data were analyzed using the analysis of variance.
*P < .001 versus group 1
*P < .01 versus group 2

Table 6. Ultrastructural data. Number of glomeruli presenting significant alterations.

	podocyte surface changes	podocyte swelling	podocyte foot process fusion
	No - %	No - %	No - %
Group 1	6 - 3.0	15 - 7.5	0 - 0.0
Group 2	187 - 93.5	165 - 82.5	189 - 94.5
Group 3	104 - 52.0	114 - 57.0	98 - 49.0
Group 4	191 - 95.5	151 - 75.5	175 - 87.0

No. = absolute number of altered glomeruli
200 glomeruli for each group were observed.

second two weeks. In group 4 the urinary volume increased as seen in group 2 (Table 4).

Protein excretion

Group 2 showed a significant increase in 24 hr protein excretion compared to group 1. In group 3 the increase of this parameter was significantly lower than that of group 2. In group 4 the protein excretion was similar to that seen in group 2 (Table 5).

Ultrastructural data

Preservation was good in control group 1 samples as seen both by SEM and TEM (Figs. 1-4).

The kidneys from group 2 showed significant and diffuse alteration of the glomeruli when compared to group 1 (Table 6). These changes were characterized by podocyte cell microvillous formation (Figs. 5, 6) podocyte cell swelling with formation of cytoplasmic blebs or vacuoles, and fusion of foot processes (Figs. 7, 8). Alterations were also present in samples treated with CPT after DXR iv.i. However, in group 3 there was a better preservation of foot processes than seen in group 4 (Figs. 9, 10). Furthermore, in group 3 the distribution of the lesion was clearly reduced when compared to the group 2 and 4 samples. In fact, as shown in Table 6, less than 60% of glomeruli in group 3 showed significant alterations, whereas 82.5 - 94.5% of glomeruli in group 2 and 87.5 - 95.0% glomeruli in group 4 had significant abnormalities.

In addition, tubular changes, characterized by an increased number of cytoplasmic droplets in proximal tubules and cast deposition in distal tubule lumen were seen in all groups, except group 1.

Discussion

The administration of DXR to rats produces severe proteinuria accompanied by progressive loss of podocyte foot processes, with swelling and vacuolization, tubular changes, and appearance of an interstitial infiltrate (Bertani et al., 1982; Hall et al., 1986; Beukers et al., 1988). DXR experimental model of renal insufficiency shows features different from those observed in rats following either five-sixth ablation of renal mass (Shimamura and Morrison, 1975) or in puromycin model of kidney disease (Mauer et al., 1972). In nephroctomized rats, ACE inhibition prevents systemic hypertension, maintains the glomerular transcapillary hydraulic pressure gradient at near normal levels, and significantly lowers proteinuria (Hall et al., 1985). Puromycin injury induces a marked increase in the mensagial uptake of macromolecules (Keane and Raij, 1985) which is normalized after administration of saralasin, an angiotensin II receptor antagonist (Raij and Keane, 1985). The pathogenesis of DXR nephropathy differs from the remnant kidney and the puromycin models of proteinuria. DXR nephrotoxicity does not seem to alter mesangial function, thus promoting mesangial overloading of macromolecules (Grond et al., 1984). In addition, since single nephron glomerular filtration rate is reduced in DXR nephrotoxicity (Michels et al., 1983), it is unlikely that RAS system has a role in the pathogenesis of the glomerular lesions induced by DXR.

Our results indicate that administration of CPT prior to the develpment of DXR nephropathy, significantly reduces albuminuria, temporarily reduces hyperdiuresis, and ameliorates the ultrastructural changes in the kidney cortex. No protective action on functional and morphological alterations induced by DXR was found when the CPT treatment started two weeks after the intravenous injection of DXR. Our findings are in agreement with previous results which demonstrated that chronic administration of CPT substantially attenuates the development of glomerular sclerosis produced in rat by DXR (Fogo et al., 1988). These workers also showed that CPT administered two weeks after DXR injection was unable to prevent nephropathy (Hall et al., 1986).

The mechanism by which CPT may slow down the development of DXR nephropathy is not understood. If the intrarenal RAS is not involved in DXR nephropathy, as discussed previously, other mechanisms must be causing the protective effect of CPT.

Several mediators, released from resident glomerular cells, reduce the size of the glomerular mesangial cells and/or their charge-selective barrier to protein. Among these mediators, platelet activating factor (PAF) and thromboxanes (TXB) seem to play a key role in inducing and/or maintaining DXR nephrotoxicity. It has been demonstrated that both PAF nephrotoxicity antagonists and TXB synthesis inhibitors, can reduce proteinuria in DXR treated rats (Remuzzi et al., 1985; Egido et al., 1987). Furthermore, it has been shown that bradykinin and prostaglandin E antagonize the local and systemic action of thromboxane (Nasyletti et al., 1975; Brenner and Schor, 1982) and CPT seems to be able to promote renal production of both kinins and prostaglandin E (Swartz et al., 1980). Therefore, it may be inferred that CPT exerts a protective effect in DXR nephropathy by increasing renal formation of both kinins and prostaglandin E. In addition, evidence has recently been accumulated suggesting the possibility that CPT acts as an oxygen radical scavenger. In fact, it exhibits anti-inflammatory properties that may be independent of ACE inhibition (Martin et al., 1984). These properties of CPT may be due to the presence of a sulphide group which in turn may scavenge deleterious free radicals (Chopra et al., 1989). Since DXR is a compound known to increase the formation of free oxygen radicals, CPT protective effect might also be due to such an activity.

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