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Effects of edaravone against glomerular injury in rats with chronic puromycin aminonucleoside nephrosis

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<Background> Excessive oxidants play roles in the pathogenesis of glomerular injury. Several studies have demonstrated protective effects of edaravone, a free radical scavenger, against renal injury. To clarify the protective effects of edaravone, we investigated changes in chemical markers of lipid peroxidation and renal histology in rats with chronic puromycin aminonucleoside (PAN) nephrosis (C-PAN).
<Methods> C-PAN was induced by intraperitoneal injections of PAN (130 mg/kg on day 1 and 60 mg/kg on day 14). Rats administered normal saline served as controls (n＝5). C-PAN rats were divided into two groups (each group: n＝4). In one group (C-PAN＋Eda), rats received 3.0 mg/kg/day of edaravone for five weeks. Blood and urinary samples were collected every week. Animals were sacrificed at the end of experiment for histological analysis.
<Results> In C-PAN＋Eda rats, urinary excretion of albumin and 8-isoPGF2α were significantly decreased compared with that in C-PAN rats ($p<0.01$), whereas levels of urinary thiobarbituric acid-reactive substances (TBARS) did not differ between the two groups. On histological examination, the scores for glomerular injury and area positive for 4-HNE were significantly lower in C-PAN＋Eda than in C-PAN rats ($p<0.05$, $p<0.01$, respectively). Infiltration of macrophages was also suppressed in C-PAN＋Eda rats.
<Conclusion> Edaravone treatment of C-PAN was able to decrease albuminuria and to ameliorate glomerular injury. Levels of several markers related to oxidative stress and lipid peroxidation were reduced after treatment, accompanied by reduction of macrophage infiltration and decrease in apoptotic cells in the glomeruli. These findings indicate that edaravone has protective effects against the glomerular injury observed in C-PAN nephrosis.
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INTRODUCTION

Recent studies have indicated that reactive oxygen species (ROS) and other oxidants play roles in the pathogenesis of glomerular injury. Single intraperitoneal administration of puromycin aminonucleoside (PAN) in rats can induce nephrotic syndrome, characterized by heavy proteinuria, hypoalbuminemia, and hypercholesterolemia. This is a well-established animal model of human minimal change nephritic syndrome (MCNS). On the other hand, the pathologic lesions of focal segmental glomerulosclerosis (FSGS) have been observed in chronic PAN nephrosis (C-PAN) induced by repeated intraperitoneal PAN injections in rats [1]. In chronic PAN nephrosis, several mechanisms related to oxidative stress may contribute to podocyte injury. It has also been proposed that hyperlipidemia may cause renal injury in this model due to the lipid peroxidation induced by ROS. Since ROS are highly reactive products of oxygen radicals, they can damage cellular components including lipids, DNA, and proteins. Thakur et al. reported [2] that treatment of PAN

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nephrosis with hydroxyl radical scavengers resulted in reduction of proteinuria.

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, MCI-186), a synthetic scavenger of free radicals, is an agent newly developed in Japan. It is a potent scavenger of hydroxyl radicals, which play a role in lipid peroxidation[3]. It has been reported to induce acute renal disorders in Japan. Post-marketing surveillance of edaravone has reported serious adverse reactions, including renal and hepatic disorders. Renal disorders were the most frequently reported serious/important adverse reactions, and this led to determination of their causation by edaravone and examination of their severity and recovery of renal function. However, a few animal and human studies on the efficacy of edaravone against chronic glomerular injury have been reported. However, these studies reported only chemical and urinary changes. The crucial issue is whether edaravone can cause renal disorder or not. The purpose of the present study was therefore to clarify whether edaravone has protective effects against proteinuria and, especially, the renal histological lesions observed in a model of C-PAN nephrosis.

Materials and methods

1. Animal experiments

This study was performed in accordance with a protocol established by the animal committee of our university. Wistar rats (five-week-old, male) were purchased from CLEA Japan Inc. and housed in a stable environment in our animal laboratory for 5 days. All animals were allowed free access to tap water and standard rat chow (CLEA Rodent Diet CE-2, CLEA Japan, Inc).

Experimental schedules were as shown in Figure 1. Nephrotic syndrome was induced by intraperitoneal injections of PAN (130 mg/kg on day 1 and 60 mg/kg on day 14). After the first injection of PAN, the rats were divided into two groups. In one group. (C-PAN+Eda rats: n=4), the rats had an infusion pump implanted (2004 ALZET Osmotic Pumps, Cupertino, CA) subcutaneously, and were continuously administered edaravone (Tanabe Mitsubishi Pharma Corporation, Japan, 3.0 mg/kg/day). This dose was three times that used clinically in humans (1 mg/kg) and sufficient for scavenging of hydroxyl radicals (company data). In the other group (C-PAN rats: n=4), the rats were administered normal saline using the same type of infusion pump. Rats administered normal saline (0.9% w/v) according to the same schedule as for the C-PAN group, and served as control (Controls: n=5).

Blood and urinary samples were collected from all the rats once a week from Week 2 to Week 5. The 24-h urinary samples were collected by placing the rats in individual metabolic cages. Body weight (BW) and renal function were estimated once a week throughout the study. All rats were euthanized at the end of the experimental period (day 35) and their kidneys were

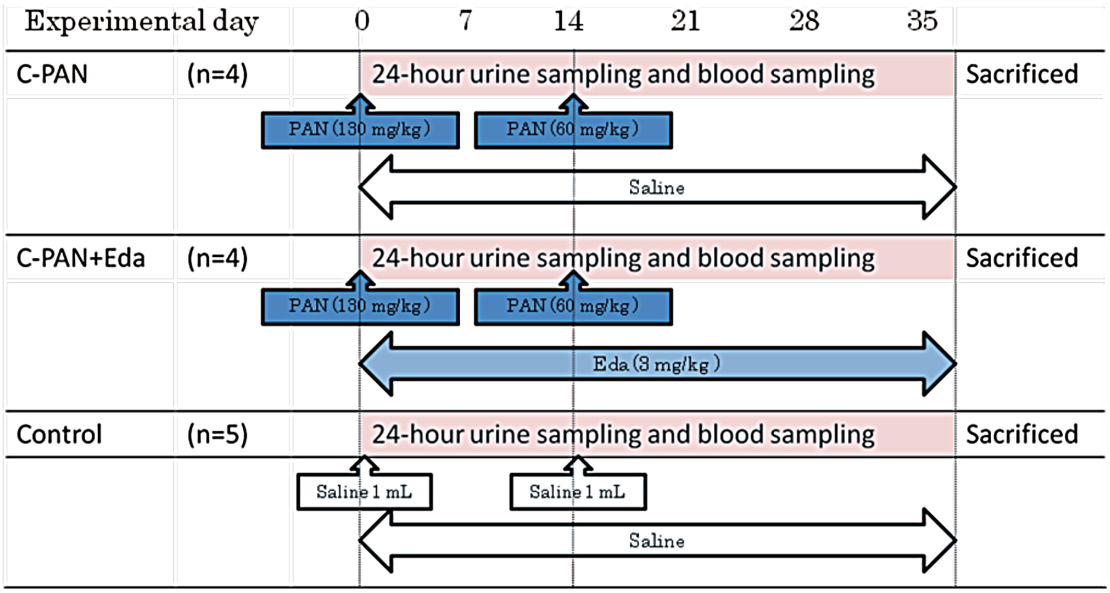

Fig. 1. The experimental protocol is shown. C-PAN: chronic puromycin aminonucleoside nephrosis without treatment; C-PAN +Eda: chronic puromycin aminonucleoside nephrosis with edaravone treatment (3 mg/kg); control: normal saline injection instead of PAN and no treatment.

removed for histological analysis.

2. Chemical analyses

The methods used to measure serum and urine levels of biochemical markers were as follows. Low-density lipoprotein (LDL), creatinine (Cr), and urea nitrogen (UN) were measured using enzymatic methods; albumin (Alb) by the BCG method; urinary albumin by nephelometry; thiobarbituric acid-reactive substances (TBARS) using hemoglobin-methylene blue[4]; and 8-epimer of prostaglandin F2alpha (8-isoPGF2α) by ultraviolet absorption spectrophotometry[5]. Urinary albumin excretion (UAE), TBARS, and 8-isoPGF2α were corrected by the urine concentration of creatinine (mg/dL). All biochemical data were obtained from day 14 to the end of the experiment (chronic phase).

3. Histological examination

A semi quantitative score (glomerular injury score: GIS) was used to assess the degree of glomerular injury according to the method of Raij et al[6]. The degree of glomerular injury in 50 glomeruli was graded using a scale of 0 to 4, based on percentage glomerular involvement; score 0: normal, score 1: lesions in up to 25% of glomeruli, score 2: lesions in 25-50%, score 3: lesions in 50-75%, and score 4: lesions in 75-100% of glomeruli. GIS was then calculated by dividing the total score by the total number of glomeruli evaluated. All tissues were stained with PAS or PAM.

4. Immunohistochemistry

After antigen retrieval, the slides were incubated with 3% H_2O_2 at room temperature and then with blocking solution (DAKO Protein Block Serum Free, Dako Cytomation Inc., Carpinteria, CA); subsequently, primary antibodies were used and Dako Cytomation EnVision＋System-HRP Labelled Polymer (Dako NorthAmerica Inc., Carpinteria, CA) was applied. After washing the sections with PBS, they were reacted with the horseradish peroxidase substrate 3, 3 diaminobenzidine (DAB, DAKO JAPAN). Hematoxylin was used for counterstaining of nuclei.

The primary antibodies used in this study were as follows: mouse anti-4-hydroxy-2-nonenal (4-HNE) antibody (NOF, Tokyo) was used for detection of a major lipid peroxidation product, and mouse anti ED-1(rat CD68) antibody (MCA341R, AbD SeroTec, Oxford, OX5 1GE, UK) was used to detect infiltrating macrophages.

In each slide, 50 randomly selected glomeruli were examined, and the area that stained positive for 4-HNE was measured using Image J Ver. 1.39 (NIH, USA). Numbers of infiltrating macrophages per glomerulus, observed as ED-1-positive cells, were counted under high-power view (×400). Data are presented as mean 4-HNE positive area/50 glomeruli and number of ED-1-positive cells/50 glomeruli.

5. Detection of apoptotic cells

For the detection of DNA fragmentation by terminal deoxynucleotidyl transferase-mediated dUTP nick-end-labeling (TUNEL) assay, sections from paraffin-embedded kidney tissue were stained using an AP *in situ* cell death detection kit (Roche, Basel Switzerland). After deparaffinization and dehydration, apoptotic cells were evaluated by TUNEL assay according to the manufacturer's instructions. In brief, the sections were incubated in proteinase K working solution (20 μg/mL in 10 mmol/L Tris/HCl, pH 7.4～8.0), and then overlaid with 50 μL TUNEL reaction mixture and incubated for 60 min at 37℃. Thereafter, the sections were overlaid with 50 μL converter-peroxidase (POD) with 100 μL diaminobenzidine substrate followed by counterstaining with hematoxylin and eosin. The percentage of TUNEL-positive cells was determined in 50 glomeruli per animal.

6. Statistical Analysis

Values are presented as the mean±standard error (SE). The paired t-test was used when two groups were compared. Simultaneous comparisons involving more than two groups were performed by analysis of variance (ANOVA) with the Tukey-Kramer test. Findings of $p<0.05$ were considered significant.

Results

1. Renal Function

In controls, serum levels of UN and creatinine remained unchanged throughout the experiment. On day 21, serum UN and creatinine concentrations were significantly increased in C-PAN and C-PAN＋Eda rats compared with controls. However, there were no significant differences between the former two groups in these parameters, as shown in Tables A and B (C-PAN, C-PAN＋Eda, vs. control: UN 72.4 mg/dL and 75.2 mg/dL vs. 21.9 mg/dL, $p<0.05$; Cr 0.49 mg/dL and 0.56 mg/dL vs. 0.28 mg/dL, $p<0.05$).

2. Serum lipid profiles

Serum levels of LDL increased and peaked on day 28 (Table C) in C-PAN and C-PAN＋Eda rats, and were significantly different from those in controls (C-PAN, C-PAN＋Eda, vs. control: 176.0 mg/dL and 184.8 mg/dL vs. 8.0 mg/dL, $p<0.05$). No significant difference in this parameter was observed between C-PAN and C-PAN＋Eda rats at any time point.

3. Excretion of albuminuria and urinary lipid peroxidation marker

The changes in UAE, TBARS, and 8-isoPGF2 α are shown in Figure 2. In both C-PAN and C-PAN＋Eda rats, UAE increased and peaked on day 21, with no significant differences between the two groups (63.0±4.7 and 56.6±2.7 mg/Cr, respectively; N.S.). The difference between C-PAN and C-PAN＋Eda rats reached statistical significance at the end of the experiment (46.4±5.3 vs. 37.1±4.5 mg/g・Cr; $p<0.05$).

Urinary TBARS also reached their maximum level in C-PAN and C-PAN＋Eda rats on day 21 (0.46±0.22 vs. 0.35±0.05 μmol/g・Cr, respectively; $p<0.05$). On the other hand, urinary 8-isoPGF2 α reached maximum level on day 28 in C-PAN and C-PAN＋Eda rats, while in C-PAN＋Eda rats it was significantly lower than in C-PAN rats (C-PAN vs. C-PAN＋Eda: 83.7±12.4 vs. 51.3±19.8 mg/g・Cr; $p<0.05$). The difference persisted until the end of the study period (48.1±8.1 vs. 28.9±2.3 mg/g・Cr, respectively; $p<0.05$). The level of urinary 8-isoPGF2 α in the C-PAN＋Eda rats on day 35 was similar to that of controls (29.9±3.9 mg/g・Cr).

4. Renal Histology

1) Light-microscopic findings: Glomerular injury score

Light-microscopic examination revealed several histological abnormalities, including segmental expansion of the mesangial matrix, obliteration of glomerular tufts, adhesion of tufts to Bowman's capsule, and regions of capillary dilatation containing foamy cells in the glomeruli of C-PAN rats. Similar findings were observed in C-PAN＋Eda rats, but they were less frequent and milder (Figure 3-B, C, D). There were no signs of global sclerosis. Moreover, GIS was significantly higher in C-PAN than in C-PAN＋Eda rats (mean GIS: 1.44±0.09 vs. 0.84±0.15, $p<0.05$) (Figure 3-E).

2) Detection of major lipid peroxidation products and oxidative stress

HNE was localized in the mesangial area and tubular epithelial cells (Fig. 4-B, C, D). Quantitative findings for staining are shown in Figure 4-E. The mean HNE-positive mesangial area was significantly higher in C-PAN rats than in controls (11.16±2.84 vs. 2.62±0.22 %, $p<0.01$). In C-PAN＋Eda rats, immunoreactivity to HNE was significantly decreased and HNE-positive mesangial area smaller than in the C-PAN (4.17±0.89%)

3) Macrophage infiltration

As shown in Figure 5, ED-1-positive cells were detected in the glomeruli of C-PAN and C-PAN＋Eda rats. Results of semiquantitative determination of the number of ED-1-positive cells per glomerulus are shown in Figure 5-E. The number of ED-1-positive cells was higher in C-PAN rats than in controls but lower than that in C-PAN＋Eda rats (4.56±0.23 in C-PAN rats, 1.17±0.07 in controls, 3.47±0.45 in C-PAN＋Eda rats, $p<0.05$).

Table Changes in concentrations of BUN (A), serum creatinine (B), and serum LDL level (C) during the experiment in C-PAN rats, C-PAN＋Eda rats, and negative control. Values are the mean ± SE. (＃$p<0.05$ vs. controls, ※$p<0.05$ vs. C-PAN rats)

A. Serum BUN Level

Experimental Days	14	21	28	35
C-PAN	30.7±11.8	72.4±37.6※	28.8±7.25	20.9±6.27
C-PAN＋Eda	27.5±16.9	75.2±2.1※	25.8±4.3	17.4±3.4
Negative Control	21.4±4.3	21.9±3.5	21.4±3.3	18.8±3.9

B. Serum Creatinine Level

Experimental Days	14	21	28	35
C-PAN	0.27±0.11	0.45±0.17※	0.34±0.10※	0.23±0.05
C-PAN＋Eda	0.31±0.06	0.56±0.06※	0.40±0.02※	0.32±0.06
Negative Control	0.28±0.05	0.28±0.03	0.28±0.02	0.28±0.03

C. Serum LDL Level

Experimental Days	14	21	28	35
C-PAN	46.6±8.6※	125.8±26.7※	176.6±54.0※	68.3±16.5※
C-PAN＋Eda	70.2±12.8※＃	134.8±8.5※	184.8±85.4※	65.8±22.3※
Negative Control	10.8±1.8	9.8±1.6	8.0±4.7	7.6±1.3

4) TUNEL-positive cells

The number of TUNEL-positive cells was significantly higher in C-PAN rats than in controls but lower than in C-PAN＋Eda rats (mean cell count: 10.88±0.34 vs. 9.17 ±0.27, $p<0.05$) (Fig. 6-B, C, D). However, there were still a substantial number of apoptotic cells in C-PAN＋Eda rats compared with the controls (mean cell count: 6.77±0.27) (Fig. 6-E).

Discussion

A single injection of PAN can induce marked proteinuria without glomerular morphological change in rats. On the other hand, repeated injection of PAN can induce massive proteinuria associated with FSGS. Previous studies suggested that lipid peroxidation was the principal cause of PAN-induced nephrotoxicity[6, 7].

In this study, we assessed renal lipid peroxidation using two parameters, the urinary concentrations of TBARS and 8-iso-PGF2. Recently, 8-iso-PGF2 has come to be considered the most reliable biochemical marker of lipid peroxidation. We found that urinary concentrations of TBARS and 8-iso-PGF2α increased after PAN injection compared with controls. However, different profiles of TBARS and 8-iso-PGF2α were observed after administration of edaravone, a potent inhibitor of lipid peroxidation[8,9]. The urinary concentration of TBARS was significantly increased on days 14 to 21 in C-PAN rats. The urinary

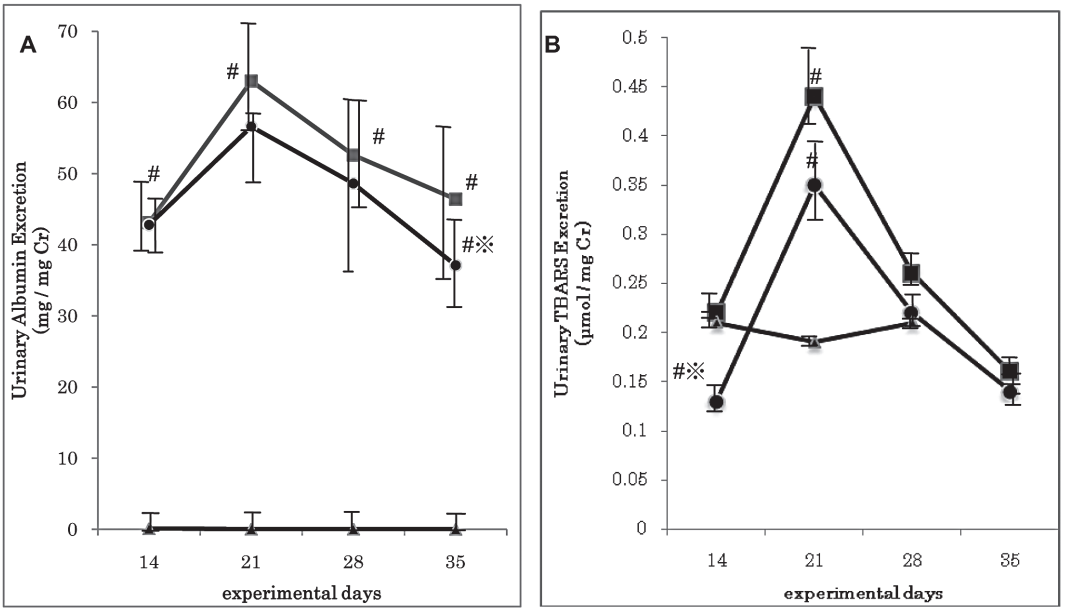

Fig. 2. Changes in concentrations of urinary albumin (A), TBARS (B), and 8-isoPGF2α (C) during the experiment in controls (▲), C-PAN rats (■), and C-PAN＋Eda rats (●).
Values are the mean±SE. (#$p<0.05$ vs. controls, ※ $p<0.05$ vs. C-PAN rats)

concentration of 8-isoPGF2 α on day 28 in C-PAN rats was significantly increased, whereas no increase in urinary 8-isoPGF2 α was observed in C-PAN+Eda rats. From these results we suggest that after PAN treatment, increasing oxidative stress levels induced further increase in urinary TBARS, and phosphatide which enriches existing in LDL surface were oxidized and it induced further increase in urinary 8-isoPGF2 α levels. In this study, edaravone treatment inhibited oxidation and resulted significant suppression of urinary TBARS and 8-isoPGF2 α levels. These findings indicated that edaravone could ameliorate urinary protein excretion in association with reduction of lipid oxidation. Similar protective effects of edaravone against several types of renal injury were reported by Matsumura et al[10]. and Someya et al[11].

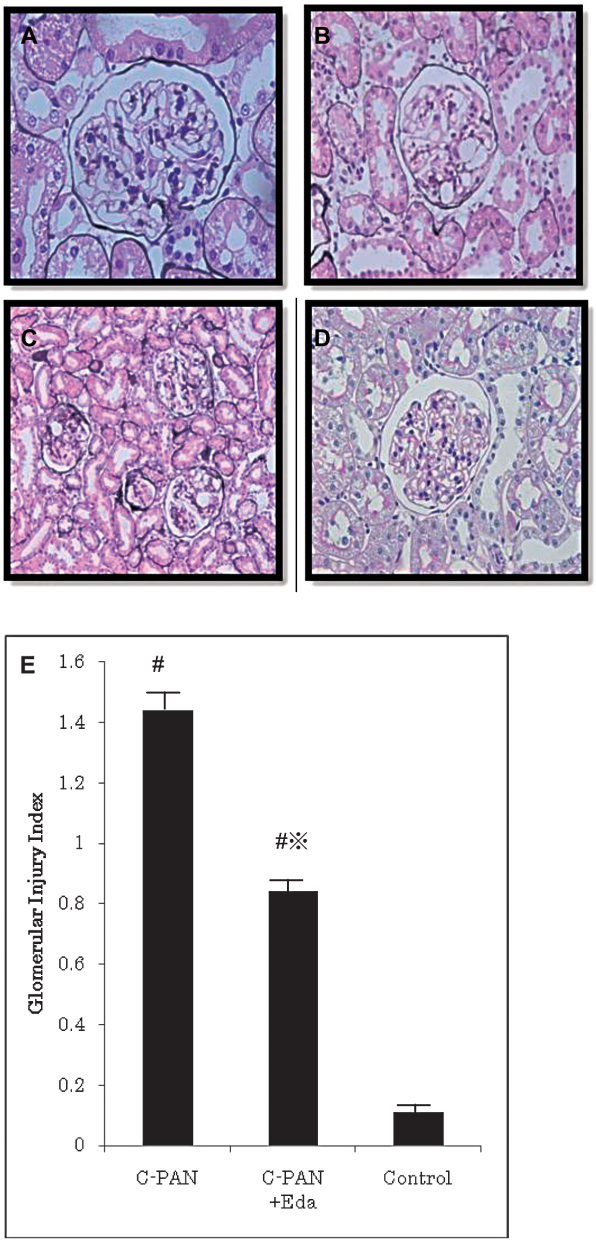

Fig. 3. Representative microscopic findings for glomerular lesions observed in PAM-stained specimens.
A: control (×400), B: C-PAN rat (×400), C: C-PAN rat (×200) and D: C-PAN+Eda rat (×400). The glomerular injury scores obtained by semiquantitative analysis are shown in panel E. Values are the mean±SE. (# $p<0.05$ vs. controls, ※ $p<0.05$ vs. C-PAN rats)

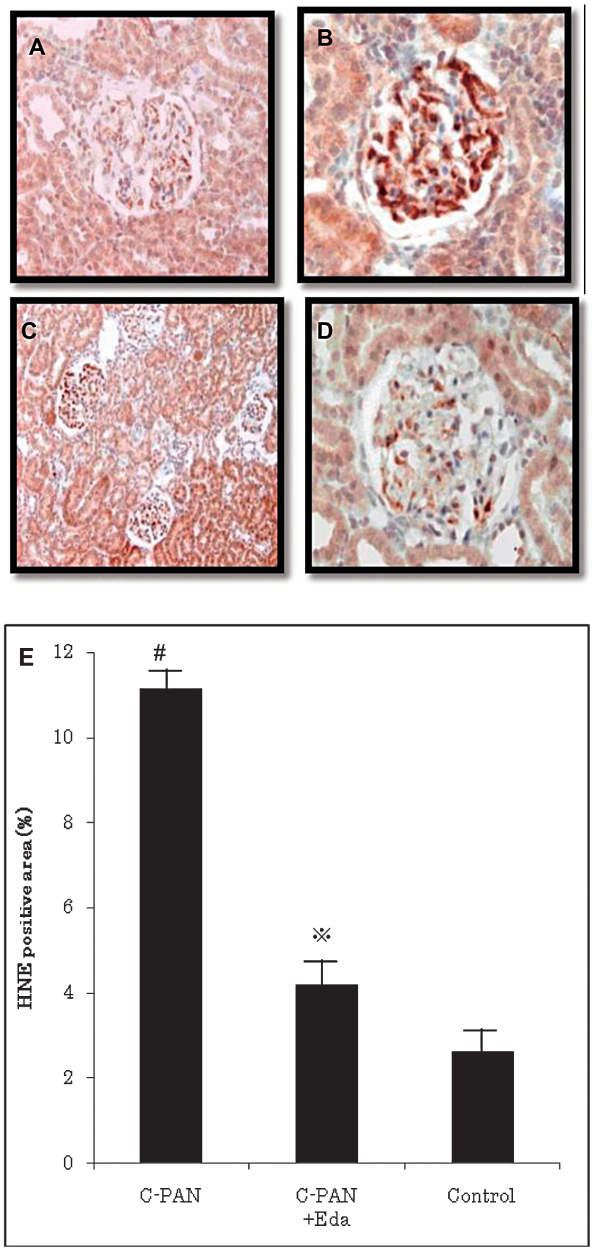

Fig. 4. Immunohistological detection of 4-HNE in a control, C-PAN rat, and C-PAN+Eda rat. Representative photomicrographs are shown in panels A~D. A: control (×400), B: C-PAN rat (×400), C: C-PAN rat (×200) and D: C-PAN+Eda rat (×400). Percent areas positively stained for 4-HNE were measured by an image analyzer and are shown in panel E. Values are the mean±SE. (# $p<0.05$ vs. controls, ※ $p<0.05$ vs. C-PAN rats)

To investigate the effects of edaravone on renal tissue injury, histological and immunohistochemical analyses were performed. The number of glomerular macrophages, as determined by ED-1 positivity, was significantly increased in C-PAN rats, as previously reported by Diamond et al[12]. Native LDL may be converted by various mechanisms to oxidized LDL (Ox-LDL), which has a specific ligand for the scavenger receptor on macrophages [13]. Oxidized LDL has been shown to be taken up by a "scavenger" receptor, distinct from the receptor responsible for the uptake of native LDL. Uptake of Ox-LDL by macrophages may further stimulate these immune effectors cells to produce growth factors, cytokines, and other mediators [14]. Infiltration of stimulated macrophages into the glomeruli may stimulate the proliferation of

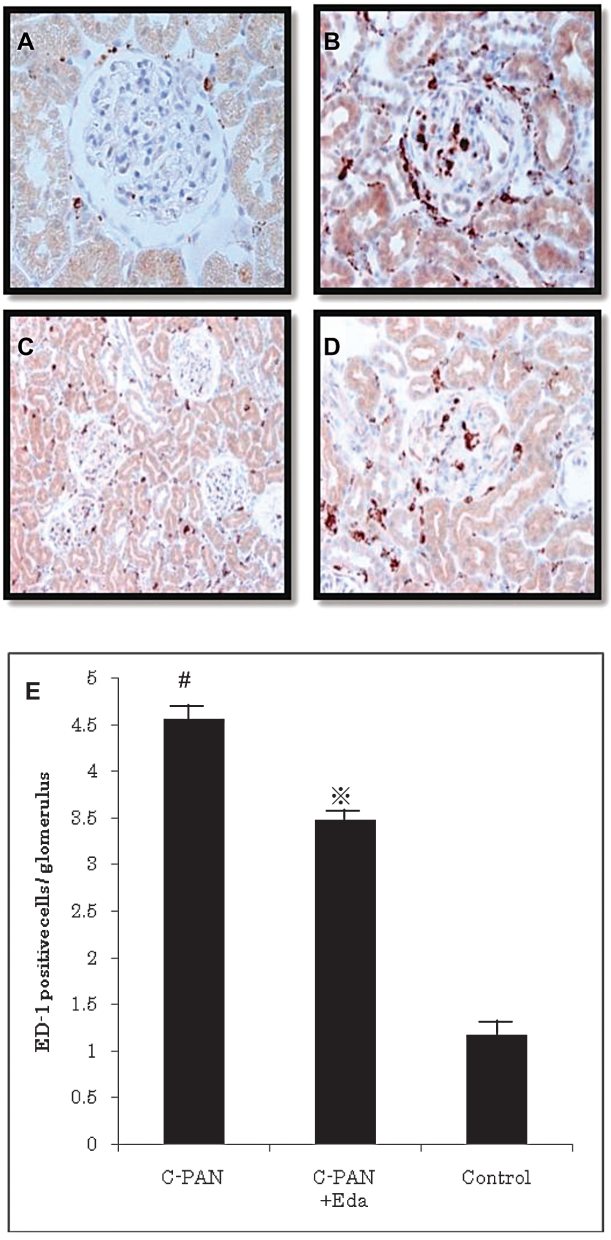

Fig. 5. Macrophages were detected by immunohistochemical staining as ED-1-positive cells. A: control (×400), B: C-PAN rat (×400), C: C-PAN rat (×200) and D: C-PAN+Eda rat (×400). Mean numbers of macrophages are given in panel E. Values are the mean±SE. (# $p<0.05$ vs. controls, ※ $p<0.05$ vs. C-PAN rats)

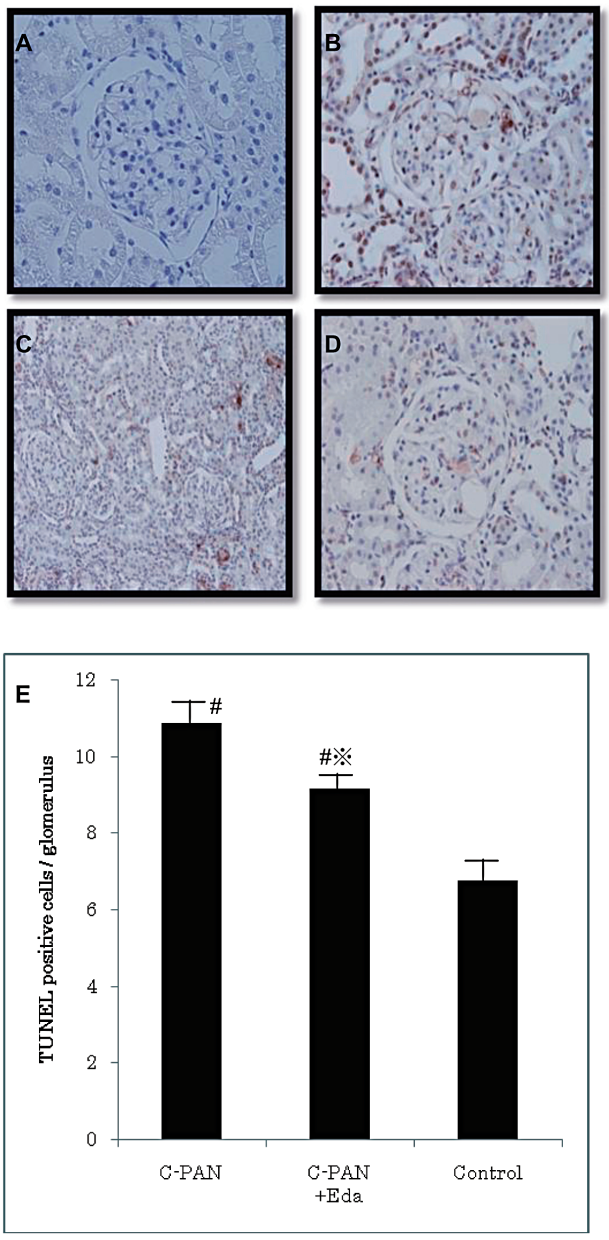

Fig. 6. Immunohistochemical detection of apoptotic cells using TUNEL assay. A: control (×400), B: C-PAN rat (×400), C: C-PAN rat (×200) and D: C-PAN+Eda rat (×400).Results of quantitative analysis of apoptotic cells are shown in panel E. Values are the mean±SE. (# $p<0.05$ vs. controls, ※ $p<0.05$ vs. C-PAN rats)

mesangial cells and extracellular matrix synthesis. During progression from the acute to the chronic phase of renal failure, the infiltrating cells change from eosinophils to monocytes/macrophages (ED-1 positive cells), or lymphocytes. This change is stimulated by monocyte chemoattractant protein-1 (MCP-1) produced by mesangial cells. MCP-1 induces worsening of glomerular lesions, leading to sclerosis and fibrosis in particular. During advanced albuminuria and renal dysfunction, ED-1-positive cells further increase with worsening of disease[15]. In the present study, treatment with edaravone resulted in significant suppression of macrophage infiltration into the glomeruli. Magi et al. reported that Ox-LDL was usually identified in the glomeruli of patients with FSGS, and that a decrease in the number of ED-1-positive cells resulted in improvement of FSGS[16]. Macrophages play key roles in the early stages of atherosclerosis and in the establishment of atherosclerotic lesions that contain abundant Ox-LDL[17]. A similar mechanism may contribute to the development of FSGS. Lipid peroxidation products, detected using antibody to HNE, were located in the mesangial area. The area positively stained for HNE was significantly reduced in C-PAN +Eda rats compared with C-PAN rats. Decreased lipid peroxidation may also reduce proteinuria and ameliorate histological changes. In the present study, the number of apoptotic cells in the glomeruli was significantly increased in chronic PAN rats, and was significantly reduced by edaravone treatment. Satoh et al. also reported that the number of apoptotic cells was significantly reduced by edaravone treatment in an experimental model of acute renal injury induced by cisplatin in rats[18]. In their study, although 1 mg/kg of edaravone did not reduce the number of TUNEL-positive cells, 5 mg/kg of edaravone significantly reduced their number. In the present study, 3 mg/kg of edaravone was used, and this dose appeared to be effective. Similarly, Iguchi et al[19]. reported that administration of edaravone reduced injury of mitochondria and DNA and renal epithelial cell apoptosis in an animal model of cisplatin-induced acute renal injury. It thus appears that the effects of edaravone may be dose-dependent and that its mode of action may involve not only the inhibition of lipid peroxidation but also direct protection of cells. Our findings suggest that edaravone scavenges ROS and free radicals generated systemically, suppresses the change from LDL to Ox-LDL.

In chronic PAN nephrosis, several mechanisms related to oxidative stress may contribute to podocyte injury. In the present study, severe albuminuria was also markedly decreased after treatment with edaravone. In addition, treatment with edaravone reduced urinary markers related to renal lipid peroxidation and improved several morphological changes. These findings indicate that renal dysfunction, as defined by increase in blood urea nitrogen, creatinine, and LDL, did not improve. However, it has not yet been reported whether edaravone improves biochemical parameters in PAN rats, and edaravone alone cannot rapidly improve renal function. Since proteinuria improved without changes in serum creatinine or urea nitrogen, this proteinuria improvement must have resulted from the radical scavenger activity of edaravone.

Edaravone has been reported to provoke acute renal disorder in Japan. However, we found no significant differences in renal function between positive control rats and edaravone-treated rats. This is important in determining whether edaravone can cause renal disorder.

The results of previous studies and our own show that edaravone has no effect on renal deficiency at the dose employed in this study. In addition, there is no report showing improvement of renal function by edaravone compared to the C-PAN model with edaravone at the dose of 3 mg/kg. In this study, it should be emphasized that whether edaravone treatment caused renal dysfunction or not. Compared with C-PAN, there were no difference about serum UN and creatinine concentration levels in the C-PAN+Eda group. From this result, we suggested that edaravone did not cause further renal function aggravation in the C-PAN group. Moreover, concerning the rise in creatinine, in a human clinical study performed from phase Ⅰ to phase Ⅲ among 549 totals, there were no report to rise in serum creatinine. Hishida et al. reported a case of renal dysfunction caused by edaravone[20,21] and no significant differences were observed in serum creatinine or urea nitrogen between control rats and edaravone-treated rats using edaravone at a daily dose of 30 mg/kg or 10 mg/kg i.v for 5 weeks[22,23]. Since aggravation of renal function was noted under conditions of infection, it appeared that the likelihood of renal dysfunction being caused by edaravone in our C-PAN rats was extremely low.

Our findings suggest that the protective effects of edaravone are probably exerted via several mechanisms. Additional studies are needed to clarify the mechanisms by which edaravone exerts protective

effects against glomerular injury.

Conclusion

The progression of renal injury observed in chronic PAN nephrosis may be related to the activation of lipid peroxidation and infiltration of macrophages into the glomeruli. In this model, treatment with edaravone resulted in a decrease in albuminuria and amelioration of glomerular injury. Histological evaluation demonstrated suppression of macrophage infiltration into the glomeruli and reduction of products of lipid peroxidation after treatment with edaravone. Over a short period of examination, antioxidant treatment thus reduced proteinuria and oxidative tissue injury. Longer observation is needed to more clearly test the effects of antioxidant treatment.

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慢性 puromycin aminonucleoside (PAN) nephrosisをもちいたエダラボン（ラジカット®）による糸球体障害への効果の検討

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≪**背景**≫種々の病態により生じる酸化ストレスにより腎糸球体は組織学的な障害を受ける．Free radical scavengerであるエダラボンによる腎障害への保護効果はいくつか報告されている．この保護作用の機序を解明するため，我々は慢性 puromycine aminonucleoside (PAN) nephrosisモデルを用いて脂質過酸化のマーカーおよび腎組織学的検討を行った．
≪**方法**≫慢性PANモデルは，PANを腹腔内投与し作成した（第1病日に130 mg/kg，腹腔内投与，第14病日に 60 mg/kg, 腹腔内投与）．ポジティブコントロール群(慢性PAN)(n＝4)，エダラボン投与群（慢性PAN＋Eda：Eda 3.0 mg/kg/day）（n＝4）の2群にグループ分けした．また，生理食塩水を腹腔内投与したwistarラット5匹をネガティブコントロール群とした．血液・尿を毎週採取し，実験終了日に麻酔下にラットより腎組織を採取した．
≪**結果**≫慢性PAN＋Eda群では慢性PAN群に比べ，尿中アルブミン量および8-isoprostane排泄量の有意な低下を認めた(p＜0.01)．TBARSは，2群間で有意な差を認めなかった．組織学的検討では，慢性PAN＋Eda群がC-PAN群に比べ，糸球体障害度，4-hydroxy-2-nonenal（4-HNE）陽性面積(p＜0.01)，ED-1陽性細胞浸潤(p＜0.05)，アポトーシス細胞数の有意な減少(p＜0.05)を認めた．
≪**結論**≫エダラボンのC-PAN群に対する投与により，尿中アルブミンを減少させ糸球体障害を改善することができた．慢性PANラットにおいて酸化ストレスおよび脂質過酸化に関連するマーカーは治療により減少し，マクロファージ浸潤および糸球体でのアポトーシス細胞の減少に相関していた．これら結果より，抗過酸化治療はC-PANにおいて糸球体障害軽減作用を有する可能性が示唆された．

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