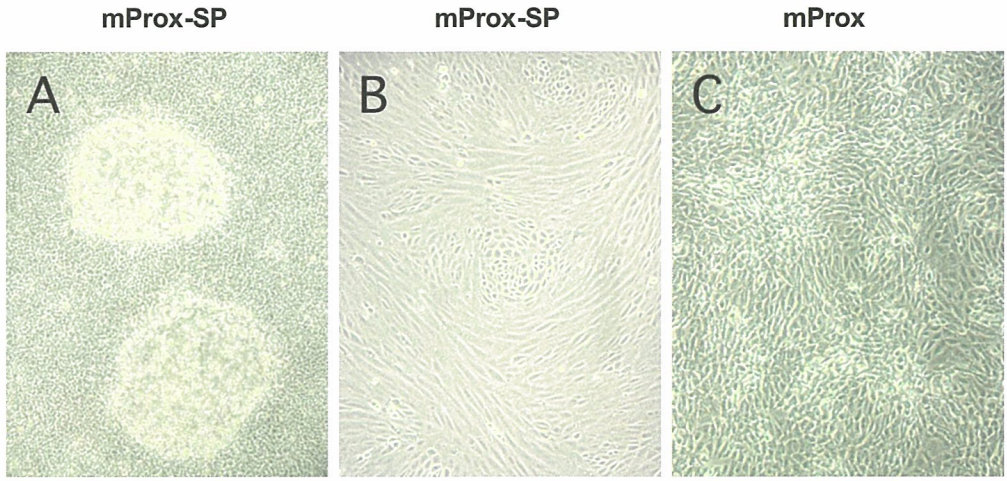
図 1


mProx-SP
mProx-SP
mProx
A
B
C

（継代培養後）

図 2

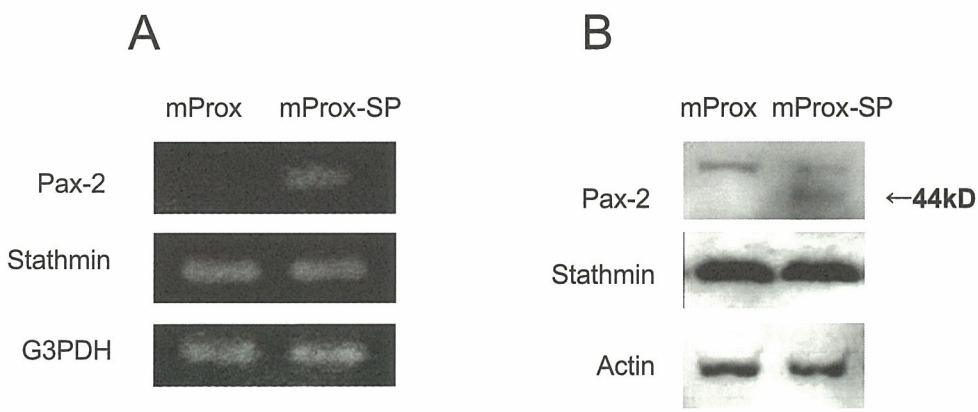
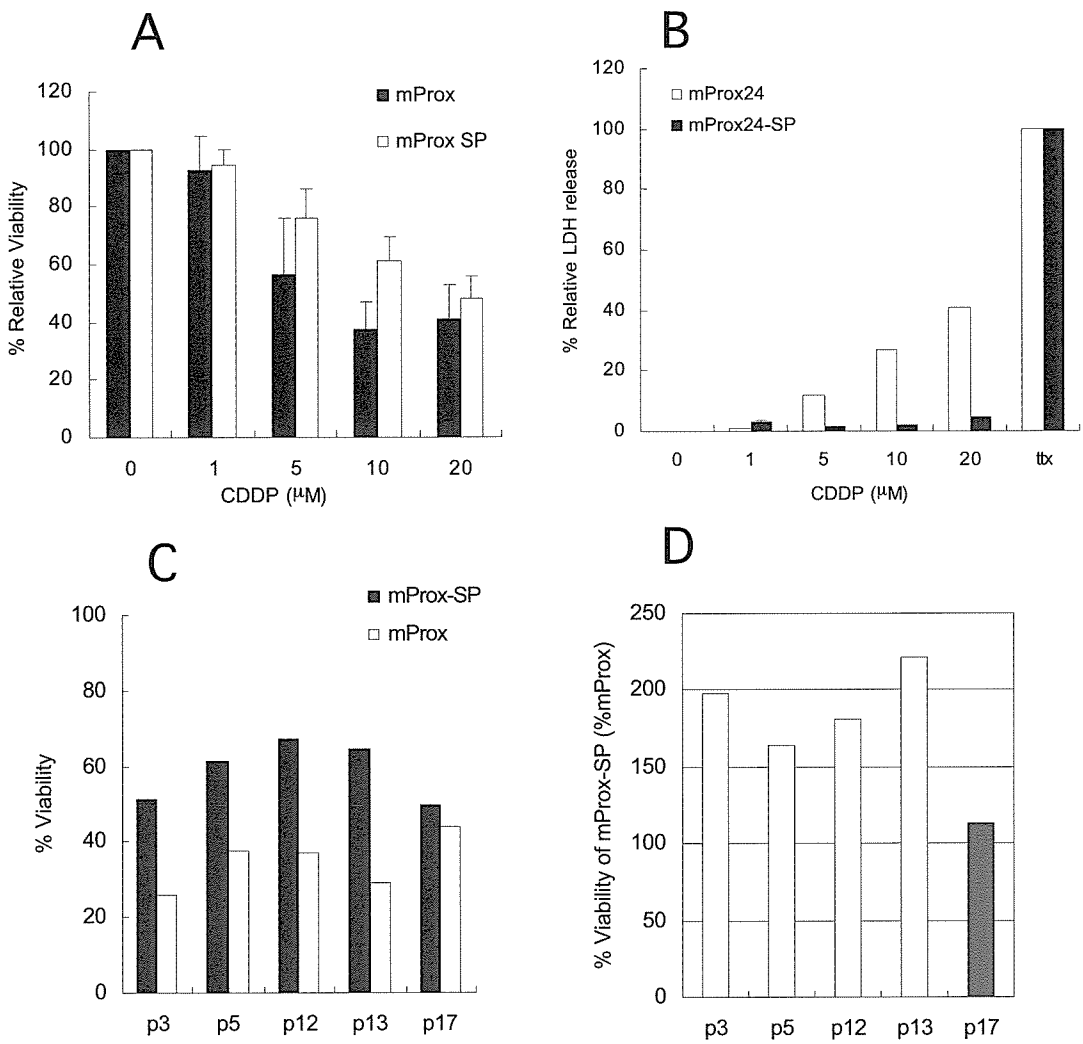

A
mProx
mProx-SP
Pax-2
Stathmin
G3PDH
B
mProx
mProx-SP
Pax-2
←44kD
Stathmin
Actin

図 3



	Passage	BUN at day3 (t-test)	Survival Rate (Kaplan-Meier)
1st	P2 – P5	p< 0.05	p< 0.05
2nd	P5 – P8	p< 0.05	N.D. (sacrificed at day5)
3rd	P21	N.S.	N.S.

図 4

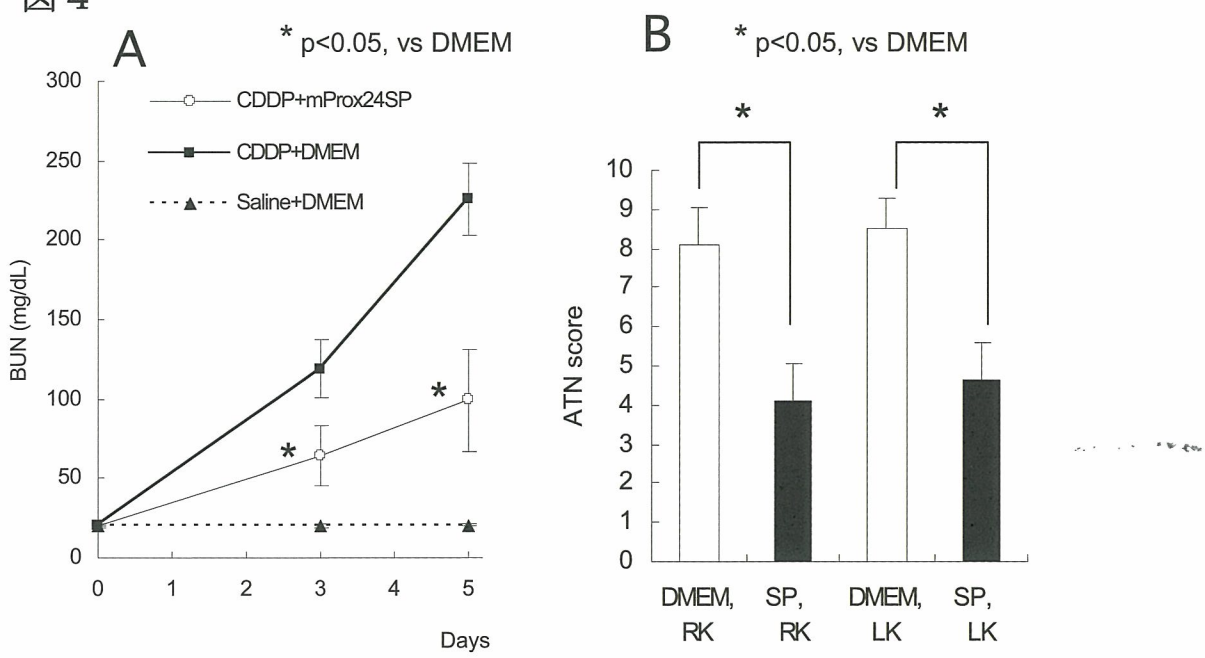

A
* p<0.05, vs DMEM
CDDP+mProx24SP
CDDP+DMEM
Saline+DMEM
BUN (mg/dL)
300
250
200
150
100
50
0
0
1
2
3
4
5
Days
*
*
B
* p<0.05, vs DMEM
ATN score
10
9
8
7
6
5
4
3
2
1
0
*
*
DMEM, RK
SP, RK
DMEM, LK
SP, LK

図 5

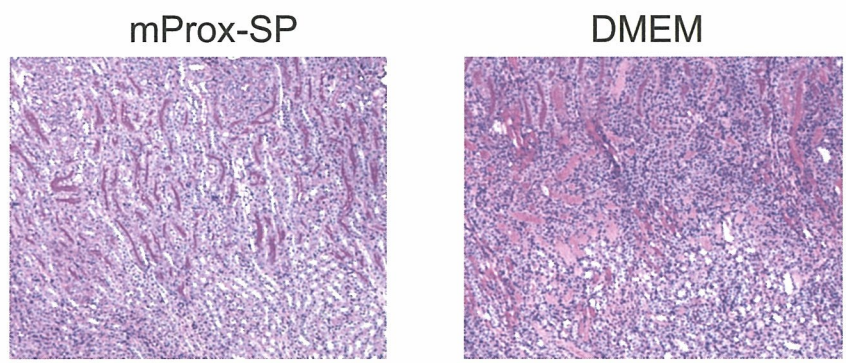
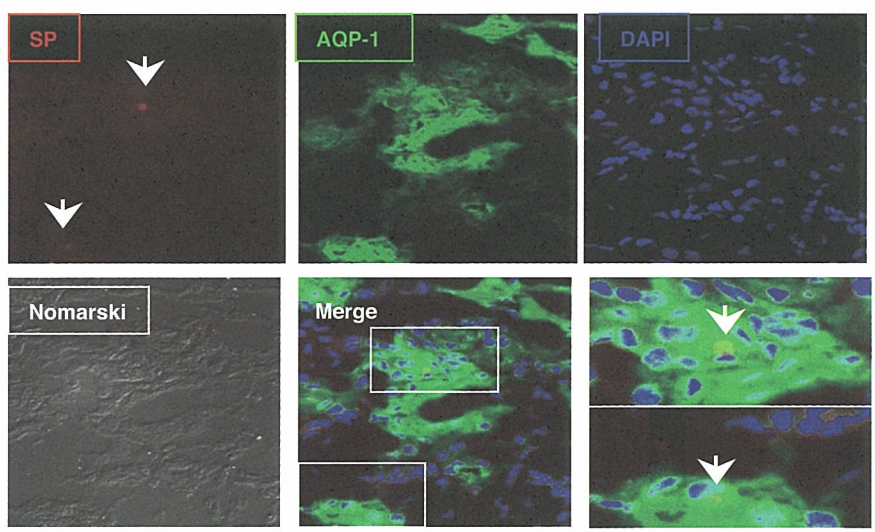

mProx-SP
DMEM

図 6


SP
AQP-1
DAPI
Nomarski
Merge

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Efficacy of Darbepoetin in Doxorubicin-Induced Cardiorenal Injury in Rats

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Key Words
Darbepoetin · Doxorubicin-induced cardiorenal injury · Rat model, cardiorenal injury

Abstract
This study was intended to elucidate the efficacy of an erythropoietin analog in cardiorenal dysfunction syndrome using a rodent model. Cardiorenal dysfunction was induced using doxorubicin hydrochloride (DXR). Lower doses (3 μg/kg) and higher doses (30 μg/kg) of darbepoetin alfa (DA) were used for intervention. Blood examinations for creatinine, blood urea nitrogen, iron, and hemoglobin were performed until 11 weeks after starting DA administration. Urine collection was performed 10 weeks after starting DA, and protein, iron, and N-acetyl-β-*D*-glucosaminidase levels and antioxidation capacity of DA were determined. The dry left ventricular heart weight was measured, when the animals were sacrificed 11 weeks after starting DA administration. Histological analyses were performed for interstitial fibrotic changes and iron deposition in the kidney. Administration of DA markedly improved anemia to the normal control level and significantly alleviated DXR-induced increases of creatinine, blood urea nitrogen, renal interstitial fibrosis, renal iron deposition, and dry left ventricular weight, but serum and urinary iron and urinary protein and N-acetyl-β-*D*-glucosaminidase levels were unchanged. The urinary total radical-trapping antioxidant capacity was improved to the normal control level in DA-treated animals. DA reduced the DXR-induced cardiorenal injury. This improvement was achieved, when anemia was corrected to the normal control level.

Introduction

In the United States, 20 million adults suffer from chronic kidney disease (CKD), and another 20 million are expected to face a heightened risk of developing CKD. An estimated 8 million adults have CKD of at least stage 3, distinguished by an estimated glomerular filtration rate <60 ml/min/1.73 m^2 [1]. Generally speaking, CKD is equivalent to progressive kidney disease towards end-stage renal disease; most patients suffer renal anemia because of insufficient erythropoietin production by the diseased kidney. Renal anemia provokes physiological abnormalities that are initially characterized by decreased oxygen delivery and utilization and increased cardiac output. These abnormalities are later characterized as left ventricular (LV) hypertrophy, decreased cognitive ability, and impaired immune response. Therefore, anemia of CKD should be treated during the predialysis period for improvement of patient survival [2] and quality of life [3]. Growing interest has centered upon the relation be-

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研究成果の刊行物・別刷

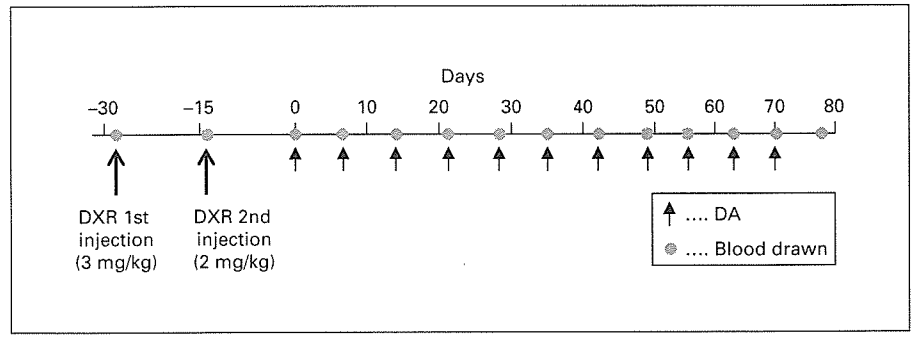


Fig. 1. Experimental protocol. Urine sampling was performed in a metabolic cage on days 71–72. The blood pressure was measured on days 75–76. Animals were sacrificed on days 77–78.

tween CKD and the risk of death, especially that caused by cardiovascular disease. Recent clinical studies [4–6] have suggested that mild-to-moderate elevations in serum creatinine (Cr) levels are associated with increased rates of death from cardiovascular causes.

Here, a question comes to our mind. If anemia in CKD is improved by erythropoietin administration, is it possible to reduce the histological deterioration in CKD and to improve renal outcome? If it were possible, then we might extend the diseased kidney's useful life span and delay initiation of dialysis therapy. Logically, we could then improve the cardiovascular outcome. Unfortunately, only limited data are available from chronic animal studies extrapolated to human CKD. Bahlmann et al. [7] clearly demonstrated the efficacy of long-acting erythropoietin, darbepoetin alfa (DA), in five-sixths-nephrectomized rats, in terms of renal tissue injury such as vascular endothelial injury, glomerular sclerosis, and tubular interstitial injury. However, renal anemia, which is common in CKD, did not develop at all in that study during the follow-up period. Bahlmann et al. [7] used a very low dose of DA (0.1 μg/kg) which did not affect hematocrit levels and did not warrant sufficient oxygen delivery to target organs.

Therefore, it is still questionable to improve the prognosis of CKD and end-stage renal disease at the clinical level. On the other hand, clinical erythropoietin therapy in CKD is intended to normalize renal anemia, but the rationale for that use to preserve other organs in addition to the kidney is still not sufficient.

For that reason, this study examined the efficacy of DA, a hyperglycosylated analog of recombinant human erythropoietin, in CKD accompanied by cardiovascular issues. We investigated doxorubicin hydrochloride (DXR) induced nephropathy in a rodent model of cardiorenal dysfunction syndrome, using either lower (3 μg/kg) or higher (30 μg/kg) doses of DA.

Materials and Methods

Materials

The DXR used for this study was purchased from Kyowa Hakko Kogyo (Tokyo, Japan). DA was produced and supplied by Takasaki Pharmaceutical Plant (Kirin Brewery, Takasaki, Japan). All other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan), unless otherwise specified. All rat experiments were approved by the Experimental Animal Ethical Committee of Kirin Brewery, in accordance with the NIH Guide for the Care and Use of Laboratory Animals [NIH Publication No. 86-23, 1985].

Experimental Design

The experimental protocol is shown in figure 1. Male 7-week-old Sprague-Dawley rats were purchased from Charles River Japan (Tokyo). They were allowed food and water ad libitum. All rats were kept in animal quarters with strictly controlled temperature (22 ± 3°C), humidity (55 ± 20%), and light conditions (lights on 08.00–20.00 h). After an acclimatization period of 11 days, the rats were assigned to the DXR group (n = 24) and the control group (n = 9); the protocol for the DXR group was performed according to our previously published report [8]. In the DXR group, the first injection of DXR at a dose of 3 mg/kg was performed 4 weeks and the second injection of DXR at a dose of 2 mg/kg 2 weeks before starting DA treatment. In the control group, the same volume (2 ml/kg) of saline was injected instead of DXR. Two different doses of DA (3 and 30 μg/kg s.c., once weekly) were used for the DXR group. Finally, 24 rats were assigned to DXR + vehicle (1 ml/kg), DXR + DA3 (3 μg/kg DA), and DXR + DA30 (30 μg/kg DA), each group consisting of 8 animals. Blood examinations were performed 4, 2, and 0 weeks before starting DA administration and subsequently 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 weeks after starting DA administration. The rats were held separately in a metabolic cage to collect urine over 24 h, 71–72 days after starting DA administration. The blood pressure was monitored 75–76 days after starting DA administration. The animals were dissected 77–78 days after starting DA administration, and kidney specimens for pathological analyses were collected after vigorous perfusion with phosphate-buffered saline and were subsequently immersed in 10% buffered formalin. The left ventricle was carefully dissected out and analzed by weight after lyophilization.

Peripheral Blood Counts

Peripheral blood was drawn from the tail artery into EDTA-2K and used for the red blood cell (RBC) count and hemoglobin (Hb) concentration measurements using an automated analyzer (ADVIA 120 Hematology System; Bayer Medical, Tokyo).

Blood Pressure

The mean blood pressure in the conscious state was measured by the indirect tail cuff method using a model MK-2000 monitor for rats and mice (Muromachi Kikai, Tokyo), according to the manufacturer's instructions. The blood pressure was recorded as the mean value of three separate measurements obtained at each session.

Blood Urea Nitrogen (BUN) and Cr

The BUN was measured using a commercial kit (BUN II reagent kit; Wako Pure Chemical Industries). Serum and urinary Cr levels were measured by means of an enzymatic assay (CRE-EN; Kainos Laboratories, Tokyo), and the Cr clearance (CCr) was calculated from a standard formula.

Urinary N-Acetyl-β-D-Glucosaminidase (NAG)

NAG is an enzyme that is rich in renal tubular epithelial lysosomes. An increase of NAG is the hallmark of tubular epithelial cell injury. Absorbance at 580 nm measures the colorimetric reaction of *m*-cresol which is generated by the hydrolytic reaction of sodio-*m*-cresolsulfonphthaleinyl with NAG [9]. This assay was commercially available, and the entire process was conducted following the manufacturer's protocol (NAG test; Shionogi, Osaka).

Serum and Urinary Iron Levels

Serum and urinary iron measurements were performed after the release of iron from its binding proteins using thioglycolic acid; Fe^{3+} was further reduced to Fe^{2+} during this reaction. Fe^{2+} reacting with 2-nitroso-5-(N-propyl-N-sulfopropylamino)-phenol generated a chelating complex that was detectable at a wavelength of 750 nm [10]. The assay kit was commercially available, and the entire process was conducted following the manufacturer's protocol (Fe C-test; Wako Pure Chemical Industries).

Urinary Protein Excretion

Urinary protein measurement was conducted during days 71–72 after starting the experiment. The urinary protein excretion was measured using a commercially available Micro TP kit (Wako Pure Chemical Industries).

Urinary Evaluation for Total Radical-Trapping Antioxidant Capacity (TRAP)

The renal antioxidant power under physiological or pathophysiological conditions consists of enzymatic and nonenzymatic antioxidative systems. Given the multiplicity of antioxidant pathways, the quantitative measurement of total antioxidant capacity will be the reasonable approach to evaluate a particular organ condition. For this purpose, the TRAP assay was performed for urine which predominantly reflects the renal antioxidant condition. In this assay, the reducing reaction of Cu^{2+} to Cu^{+} stably generates a 2:1 complex with the chromogen reagent and is measurable at approximately 490 nm using a plate reader. This colorimetric assay was used according to the manufacturer's protocol (TA 01; Oxford Biomedical Research, Oxford, Mich., USA).

Histological Analysis

The kidneys were embedded in paraffin (TissuePrep; Fisher Scientific, Pittsburgh, Pa., USA) and cut into 3-μm sections. After sequential dewaxing and rehydration, the sections were stained with periodic acid-Schiff, Masson's trichrome, and Berlin blue. Histological images were taken by means of a digital CCD camera (DXM 1200F; Nikon, Tokyo) and examined using an Optiphoto-2 microscope (Nikon).

Morphological Evaluation of Kidneys

The area of interstitial fibrosis in the cortex was evaluated with Masson's trichrome using a computer-aided evaluation program (AIS Ver4.0; Fujifilm, Tokyo). Under ×400 magnification, five randomly selected nonoverlapping fields from the cortical region were analyzed. The fibrotic areas that were stained in blue were picked up on digital images, and the percentage of the fibrotic area relative to the whole area of the field was calculated (% area). Glomeruli and large vessels were not included in the microscopic fields for image analyses. The scores of each kidney were averaged. The scores of respective animals were then averaged.

The iron deposition in Berlin blue staining was evaluated semiquantitatively in a blind fashion without prior knowledge of the rat group. At least ten fields were examined at ×400 in each specimen, and the iron staining of the lesion was graded from 0 to 4+, according to the percentage of Berlin blue staining. Consequently, a 1+ lesion represented an involvement of approximately 5% of the field, a 2+ lesion had involvement of 5–15%, a 3+ lesion indicated an involvement of 15–25%, and a 4+ lesion had an involvement >25% of the field. Each field represents 1.13 mm^2, resulting in a total explored area of 11.3 mm^2 in each kidney.

Statistics

The results are expressed as mean values ± SEM. Differences among experimental groups were determined using one-way ANOVA with Fisher's post hoc analysis. Differences with $p < 0.05$ were considered significant.

Results

Both RBC and Hb values in the DXR group decreased significantly as compared with those of the DA-treated animals during the follow-up period (fig. 2). The level of anemia was normalized to that of the control group, when DA at 3 μg/kg was administered to DXR animals (DXR + DA3). The level of anemia was further improved in DXR animals that received 30 μg/kg of DA (DXR + DA30). The levels of RBC and Hb were significantly increased as compared with the control group. The time courses of BUN and Cr are summarized in figure 3. Both BUN and Cr levels increased gradually in the DXR group 6 weeks after starting DA administration. The levels of BUN and Cr in the DXR group were markedly reduced in the DA-treated animals. This improvement was not different for the animals treated with high and low doses of DA. Similarly, the CCr in the DXR group was signifi-

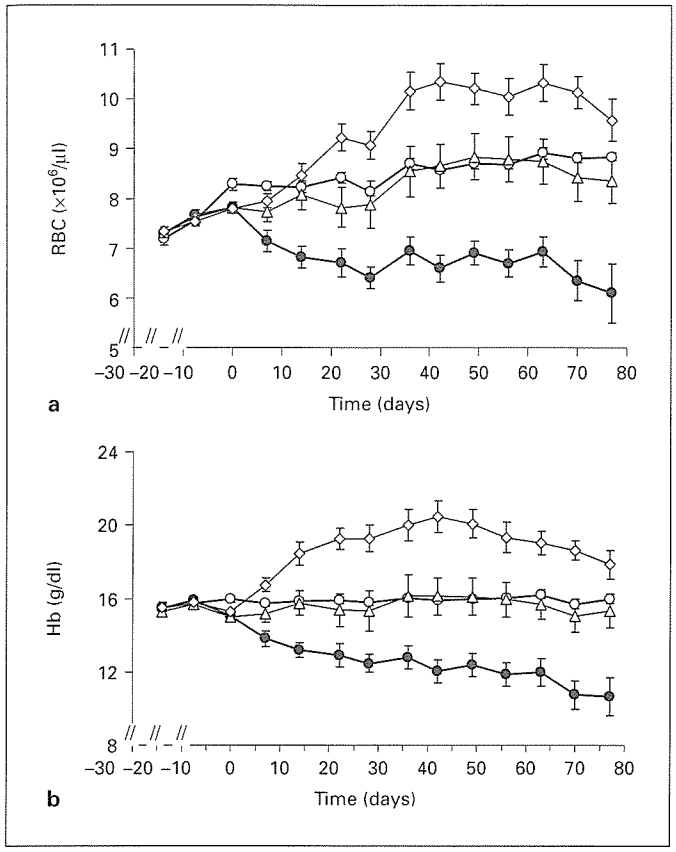


Fig. 2. Time courses of RBC (**a**) and Hb (**b**). ○ = Controls (n = 9); ● = DXR-treated animals (n = 8); △ = DXR + DA3 (low dose of DA; n = 8); ◇ = DXR + DA30 (high dose of DA; n = 8).

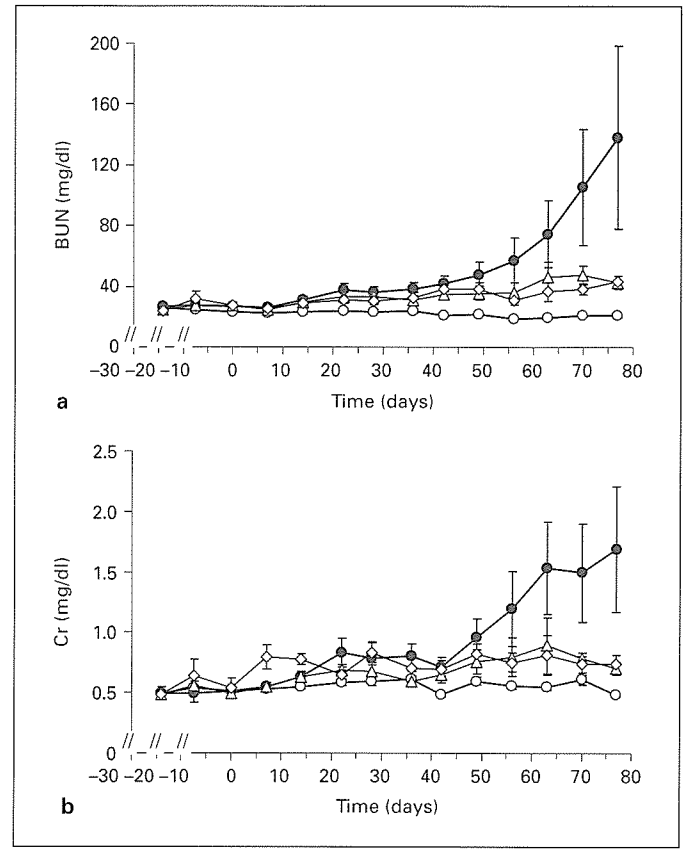


Fig. 3. Time courses of BUN (**a**) and Cr (**b**). ○ = Controls (n = 9); ● = DXR-treated animals (n = 8); △ = DXR + DA3 (low dose of DA; n = 8); ◇ = DXR + DA30 (high dose of DA; n = 8).

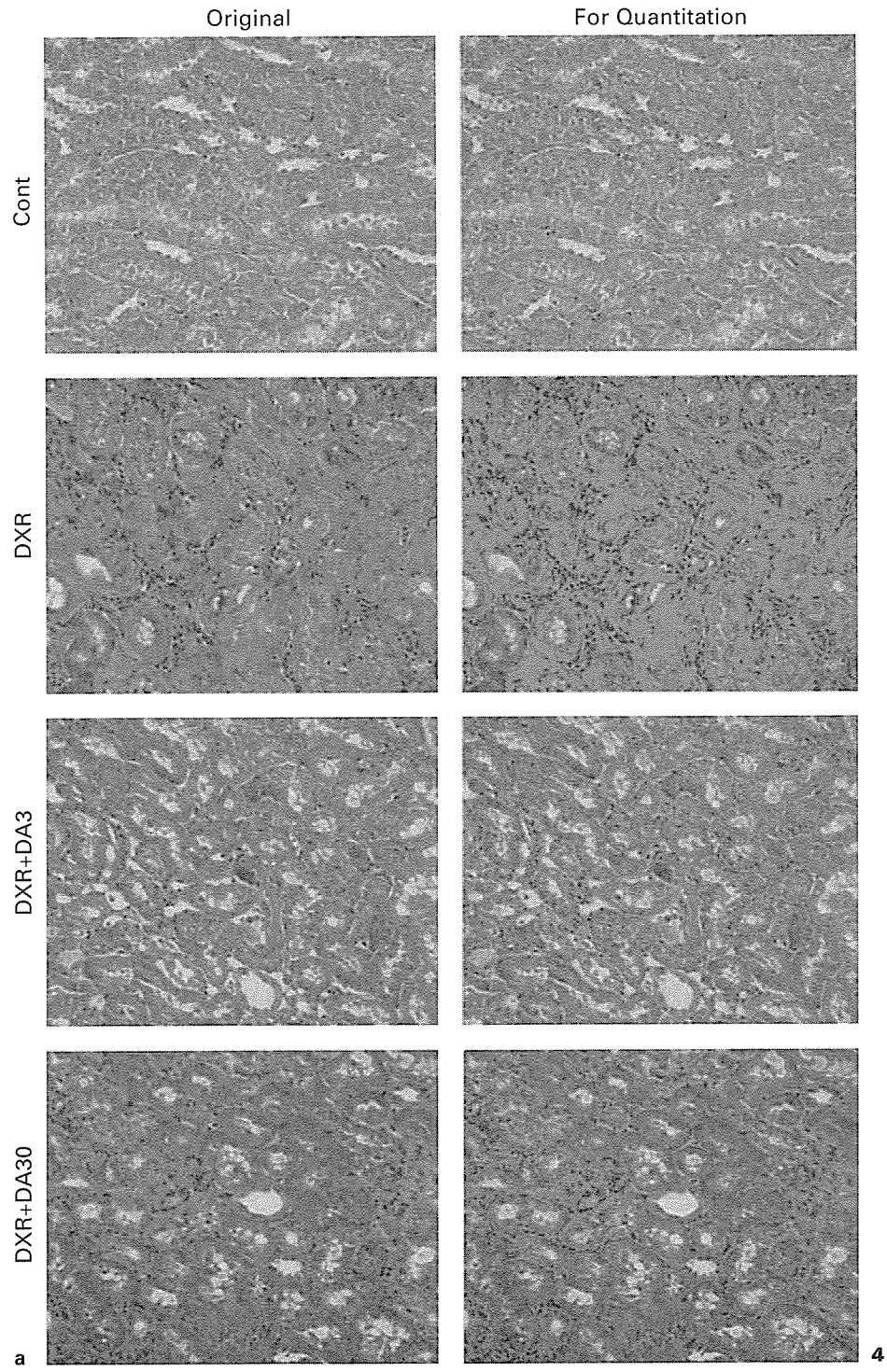
cantly decreased to 0.75 ± 0.22 ml/min as compared with the control group showing 2.15 ± 0.15 ml/min. This decrease in the CCr was improved in both animal groups treated with the low dose of DA (1.22 ± 0.10 ml/min) and with the higher dose of DA (1.32 ± 0.13 ml/min).

The mean blood pressure 75–76 days after starting the experimental protocols was 93.1 ± 8.8 mm Hg in the control group, 97.9 ± 12.4 mm Hg in the DXR group, 106.5 ± 9.3 mm Hg in the DXR + DA3 group, and 113.1 ± 8.6 mm Hg in the DXR + DA30 group. A tendency towards an increased mean blood pressure was apparent in the DA-treated groups, but this increase did not reach statistical significance.

Quantitative analysis of interstitial fibrosis was performed using Masson's trichrome staining. The severity of peritubular fibrosis was prominent in the kidneys of the DXR (DXR kidneys), as seen in figure 4a. The proportion of the fibrotic area (summarized in figure 4b) from the ×400 field was 36.43 ± 1.27% (n = 40 fields from eight kidneys) in the DXR kidneys as compared with 3.08 ± 0.34% (n = 45 fields from nine kidneys) in the control kidneys. This latter proportion was significantly lower for both doses of DA. The proportions of fibrotic areas were 7.59 ± 0.45% (n = 40 fields from eight kidneys) in the DXR + DA3 group and 7.51 ± 0.64% (n = 40 fields from eight kidneys) in the DXR + DA30 group. It is noteworthy that iron deposition represented by Berlin blue staining (fig. 5) was apparent in pericapillary interstitium and tubular basement membrane in the renal cortical region obtained from the DXR-treated animals. On the other hand, DA administration together with DXR reduced blue staining. Fine staining was, however, observed in proximal tubular cells in DXR kidneys treated with DA. Semiquantitative analyses indicated a significant increase of iron deposition in DXR animals. It was reduced to a statistically significant level in DA-treated kidneys in ani-

mals that received both the lower and the higher dosage ($p < 0.05$; fig. 5).

The serum iron level was decreased in the DXR group and in the DXR groups treated with with DA (DXR, 92.7 ± 10.9 μg/dl; DXR + DA3, 105.5 ± 11.0 μg/dl, DXR + DA30, 108.4 ± 15.8 μg/dl; n = 8 in each group) as compared with the control group (185.3 ± 5.1 μg/dl, n = 9) 77–78 days after starting DA administration. The urinary iron level was remarkably elevated in both the DXR group and the DXR groups treated with DA (DXR, 171.7



a 4

± 34.2 μg/kg; DXR + DA3, 214.8 ± 26.4 μg/kg; DXR + DA30, 223.5 ± 11.6 μg/kg) as compared with the control group (12.5 ± 1.8 μg/kg). The same phenomenon was found for the level of proteinuria (20.5 ± 1.8 μg/kg; DXR, 1,356.9 ± 192.4 μg/kg; DXR + DA3, 1,693.0 ± 214.0 μg/kg; DXR + DA30, 1,797.1 ± 159.6 μg/kg).

The level of urinary transferrin excretion is dependent on the level of proteinuria. Therefore, the ratio of urinary iron to proteinuria was calculated and is shown in figure 6. No significant difference was apparent between DXR groups with or without DA treatment. Therefore, the administration of DA per se did not promote iron excretion in this rodent CKD model.

The level of urinary NAG was significantly higher in the DXR-treated animals (2.96 ± 0.42 U/kg) than in the controls (0.78 ± 0.04 U/kg). No significant difference was apparent between DXR groups receiving DA (DXR + DA3, 3.05 ± 0.38 U/kg; DXR + DA30, 3.19 ± 0.49 U/kg).

The urinary measurement of TRAP (fig. 7) 71–72 days after starting DA administration revealed a significantly lower level in the DXR group (152.8 ± 18.2 μmol/kg) than in the control group (174.9 ± 16.7 μmol/kg). On the other hand, the TRAP level was significantly improved and even increased in the DXR-treated animals receiving both the lower DA dose (DXR + DA3, 214.0 ± 16.2 μmol/kg) and the higher DA dose (DXR + DA30, 240.2 ± 12.8 μmol/kg) as compared with the control or DXR-alone groups.

Finally, the index of the DXR-induced LV hypertrophy, represented as dry heart weight, is summarized in figure 8. The LV weight was significantly higher in the DXR group (592 ± 48 μg/kg; n = 8) as compared with

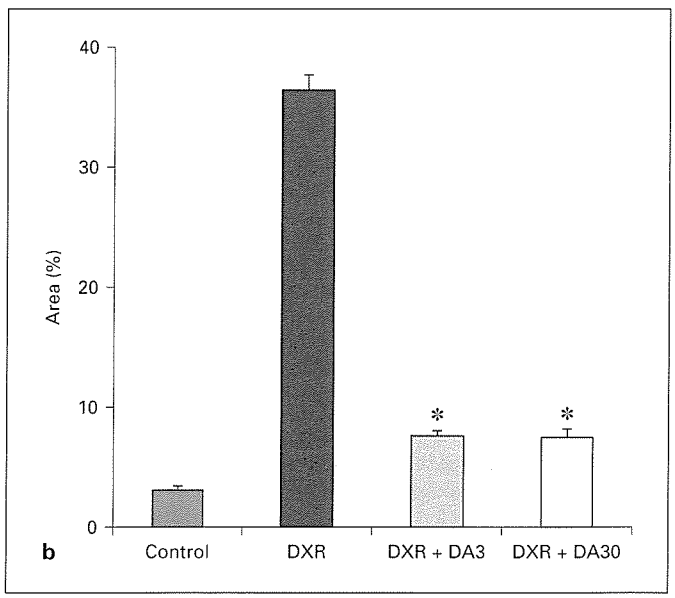


Fig. 4. a Representative images for renal interstitial quantitation. The level of blue color in Masson's trichrome staining (original) was gauged by image analysis software AIS and is expressed as light green in the right column. Glomeruli, tubular lumen, and large vessels were excluded from the microscopic fields. **b** The percentage of fibrotic areas relative to the whole area of the field was calculated. Asterisks indicate statistically significant difference when compared with the DXR-treated group ($p < 0.05$)

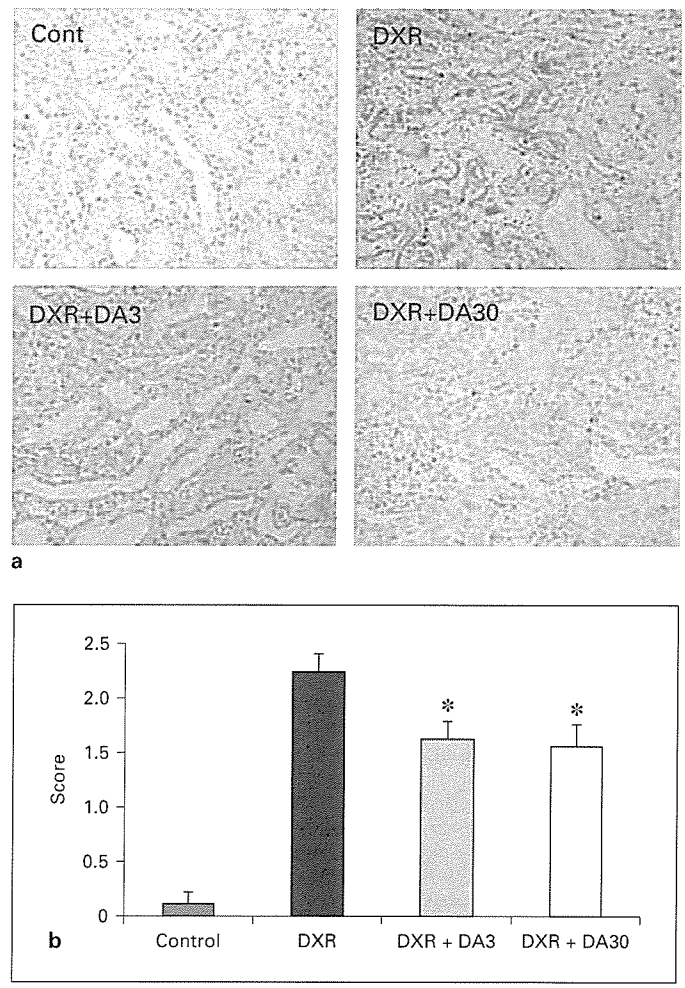


Fig. 5. Representative images of Berlin blue staining for iron deposition (**a**) and semi-quantitative analyses (**b**). Cont = Control. A 1+ lesion represented an involvement of approximately 5% of the field, a 2+ lesion an involvement of 5–15%, a 3+ lesion an involvement of 15–25%, and a 4+ lesion indicated involvement of more than 25% of the field. The asterisks in **b** indicate statistically significant difference when compared with the DXR-treated group ($p < 0.05$).

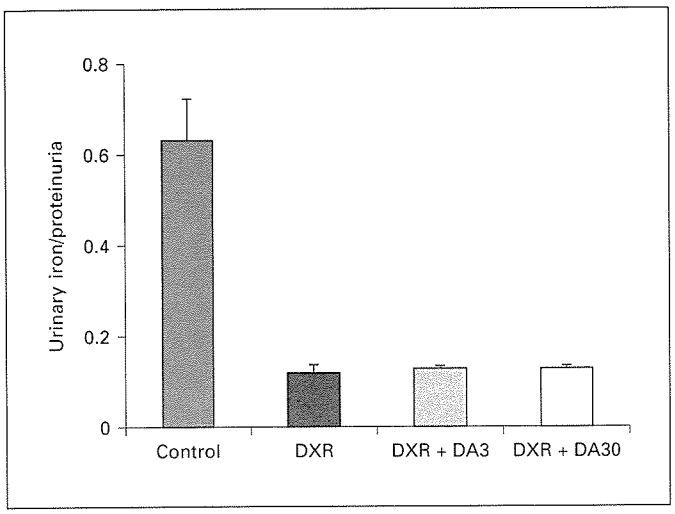


Fig. 6. Ratio of urinary iron excretion to proteinuria. Urine sampling was performed on days 71–72. The control group consisted of 9 animals, all others of 8 animals.

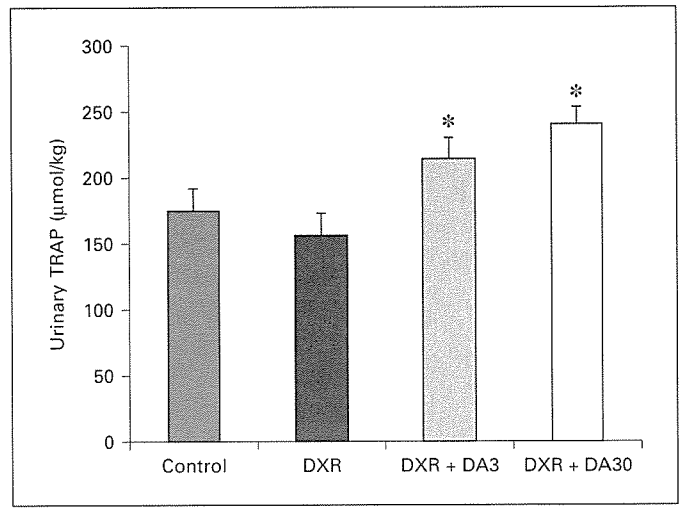


Fig. 7. Total urinary TRAP. Urine sampling was performed on days 71–72. The control group consisted of 9 animals, all others of 8 animals. Asterisks indicate statistically significant difference when compared with the DXR-treated group ($p < 0.05$).

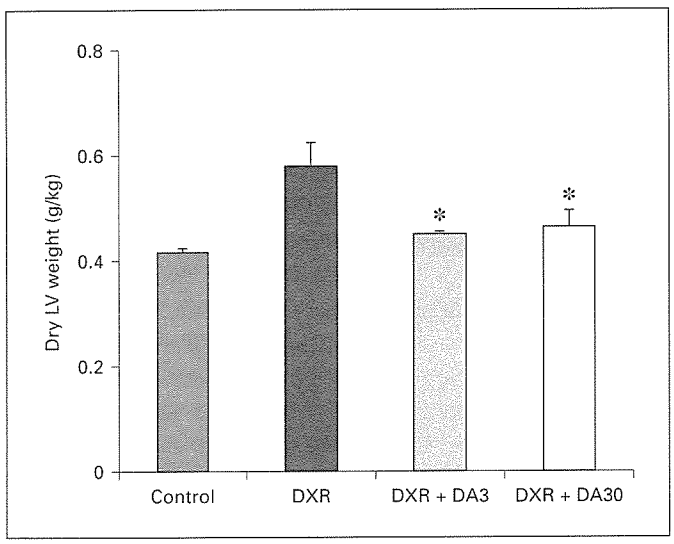


Fig. 8. Dry LV weight. The animals were dissected 77–78 days after starting DA administration. The control group consisted of 9 animals, all others of 8 animals. Asterisks indicate statistically significant difference when compared with the DXR-treated group ($p < 0.05$).

that of the control group (415 ± 8 μg/kg; n = 9). This increase was significantly ameliorated in the DA-treated animals receiving either the lower DA dose (DXR + DA3, 449 ± 7 μg/kg; n = 8) or the higher DA dose (DXR + DA30, 435 ± 9 μg/kg; n = 8).

Discussion

The prevalence of anemia increases according to the stage of CKD, as addressed in recent K/DOQI guidelines [11]. Very few CKD individuals are anemic at stage III disease: about 15%. However, by stage IV, when the glomerular filtration rate is in the 15- to 29-ml/min/1.73 m^2 range, about 50% of the patients develop some degree of anemia. Several studies have reported that the level of the CCr is associated with adverse cardiovascular outcomes, particularly in patients with coronary artery disease [4, 6, 12–15]. In addition, a higher prevalence of renal insufficiency was reported in patients with heart failure. Multivariate analyses [16] of the RENAAL study [17] demonstrated that renal disease itself was an independent risk factor for developing cardiac disease just as hypertension, hyperlipidemia, smoking, and diabetes are. Furthermore, cardiac patients with anemia have worse outcomes; therapy of anemia improves their outcomes. If CKD, anemia, and cardiac disease share a triangular relationship, does treatment of anemia somehow forestall progression of CKD and cardiac disease? To answer this question, we have chosen DXR as a nephrotoxic and cardiotoxic agent in this study.

The DXR protocol used in this study clearly induced renal function deterioration, as demonstrated by BUN, Cr, and CCr. Correction of anemia to the level of the control group remarkably preserved renal functions; this

efficacy was equivalent for both the DXR group with a lower dose of DA and the DXR group with a higher dose of DA. The tubular injury marker NAG was not different between DXR-alone animals and DXR animals treated with lower or higher DA doses at days 71–72. Also, NAG did not reflect the differences observed in BUN, Cr, and CCr as a tubular injury marker in later stages of CKD. Histologically, the DXR kidneys showed tremendous fibrotic changes of the cortical tubular interstitium together with tubular atrophy and progressive scarring in the interstitium. These changes found in DXR kidneys were improved, when the animals were treated subsequently with either lower or higher doses of DA. These differences were quantitated using image analysis software to determine the statistically significant improvement of DA-treated kidneys over DXR kidneys. The dose of DA, either lower or higher, does not reflect this improvement. More intriguingly, DXR animals demonstrated more Berlin blue iron staining in their kidneys, especially at the cortical tubular basement, as shown in figure 5. This was reduced when DA was given to DXR animals. Next, we evaluated serum and urinary iron levels. The serum iron levels were reduced in all DXR animals, with or without DA treatment, probably due to urinary loss of transferrin together with proteinuria. No difference was apparent for the effect of DA on urinary iron levels in these animals. Therefore, the decrease of peritubular Berlin blue iron staining found in DA-treated DXR animals is presumably related to the increased hematopoiesis induced by DA which is, therefore, at least in part, able to decrease the level of tubular damage. This aspect was monitored by the urinary TRAP level. The TRAP level was rather more defective in DXR animals than in controls; it was significantly improved in DA-treated animals receiving either low or high doses. The improved findings observed in DA-treated kidneys were presumably related to chronic tubular rather than glomerular damage. These findings, therefore, support the concept that tubules and interstitium play a pivotal role in progressive kidney disease and are more predictive of the renal outcome. The oxygen tension of tubules is lower than that of glomeruli; tubules were more susceptible to hypoxic injury mediated by reactive oxygen species [18]. Treatment with erythropoietin will increase the oxygen delivery to these tissues, thereby reducing the risk of hypoxic injury.

DXR is a well-known cytotoxic drug causing cardiomyopathy, but the DXR dosage used in this study was far below that frequently used for the induction of DXR cardiomyopathy in rats [19]. Therefore, we believe that the factor related to diffuse cardiac fibrosis would be minimal in this study, but further histological studies are necessary. Anemia often promotes LV hypertrophy, and normalization of anemia by erythropoietin in predialysis CKD patients improved LV hypertrophy using the LV mass index as an indicator [20, 21]; this study elucidated this add-on effect. The LV dry weight, an alternative to the LV mass index in animal studies, was not increased in the present study, when the effect of anemia was improved in the DA groups receiving either low or high doses. These animal data further support results of a preceding human study reporting a 32% increased risk of a cardiovascular event for every 0.5-g/dl decrease in the Hb level in predialysis patients [22] and the finding that CKD patients with Hb levels <11.2 g/dl are three times more likely to progress to end-stage renal disease than those with Hb values >13.8 g/dl [16]. The observed efficacy of DA in DXR-induced LV hypertrophy will be partially derived from the direct effect of DA through erythropoietin receptors in cardiomyocytes, as seen in recent reports on rat ischemic heart models [23, 24], in addition to the indirect effect of DA: the increase of oxygen delivery to cardiomyocytes by correction of anemia. The erythropoietin-induced organ-protective effect against cardiorenal syndrome was achieved using a DA dose of 3 μg/kg which is sufficient to maintain a normal Hb level. The significant increase of the Hb levels beyond the normal control values was not important, because we were unable to detect further improvements in the parameters of our current study.

This animal study clarified the efficacy of DA in CKD and LV hypertrophy, supporting the clinical observations using erythropoietin reported by Gouva et al. [25]. In their report, patients who were treated with erythropoietin exhibited a dramatic increase in their Hb levels from 10 g/dl to almost 13 g/dl, whereas patients who were not treated showed no changes in Hb levels. Even though both groups had some decrease in their kidney function, this decrease was much more dramatic in patients that were not treated. Moreover, when these authors looked at their primary end points, doubling of serum Cr levels or progression to end-stage renal disease, patients who were not treated for their anemia had double the risk towards these end points. Based on these clinical data and the results of our study, correction of anemia to the normal level by erythropoietin or DA retards the progression of CKD and cardiovascular diseases.

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