Activation of hypoxia-inducible factor attenuates renal injury in rat remnant kidney

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Abstract

Background. Chronic hypoxia in the kidney has been suggested as a final common pathway to end-stage renal disease. Hypoxia-inducible factor (HIF) is a transcription factor that regulates cellular hypoxic responses, and it is a promising target with therapeutic potential in various kidney disease models. In this study, we investigated whether HIF activation could attenuate renal injury in the rat remnant kidney model.

Methods. Two weeks after a subtotal nephrectomy, rats received a continuous infusion of dimethyloxalylglycine (DMOG) for 4 weeks to activate HIF.

Results. The DMOG infusion halted the progression of proteinuria. A histological evaluation revealed that the glomerulosclerosis and tubulointerstitial injury were significantly decreased by DMOG treatment. DMOG increased renal HIF-1α protein. The expression of glucose transporter-1 (GLUT-1) and prolyl hydroxylase 3 (PHD3) and the immunostaining of vascular endothelial growth factor (VEGF) were increased by DMOG. DMOG-treated rats showed less podocyte injury manifested by decreased immunostaining of desmin and the restoration of podoplanin staining. Furthermore, plasma malondialdehyde (MDA), a marker of oxidative stress, showed a tendency to decrease, and the renal expression of catalase, an antioxidant, was significantly increased by DMOG. The DMOG treatment decreased macrophage infiltration and reduced fibrosis, as manifested by decreased type IV collagen and osteopontin expression.

Conclusions. Activation of HIF by DMOG halted the progression of proteinuria and attenuated structural damage by preventing podocyte injury in the remnant kidney model. This renoprotection was accompanied by a reduction of oxidative stress, inflammation and fibrosis.

Keywords: DMOG (dimethyloxalylglycine); fibrosis; inflammation; oxidative stress; podocyte injury

Introduction

Most cases of chronic kidney disease inevitably progress to end-stage renal disease, which has a high associated morbidity and mortality. Although the initiating insult of chronic kidney disease is variable, the progression of the disease seems to be common to all kidney diseases that involve a vicious cycle of nephron destruction, glomerulosclerosis and tubulointerstitial fibrosis [1]. The kidney is sensitive to changes in oxygen delivery and prone to hypoxic injury. Recent studies emphasize the role of chronic hypoxia in the kidney as a final common pathway to end-stage renal disease [2–5]. Therefore, therapeutic intervention against hypoxia can be a valid tool to halt the progression of chronic kidney disease.

Hypoxia-inducible factor (HIF), a basic helix-loop-helix transcription factor composed of an oxygen-sensitive α subunit and a constitutively-expressed β subunit, is an important regulatory factor that allows individual cells to adapt to hypoxia [6]. In normoxia, the α subunit rapidly undergoes prolyl hydroxylation, interaction with the von Hippel–Lindau protein and proteasomal degradation [7–10]. In hypoxia, the α subunit escapes prolyl hydroxylation and binds to the β subunit, and the heterodimeric HIF translocates to the nucleus and activates the transcription of genes involved in erythropoiesis, angiogenesis, cell metabolism, cell growth and apoptosis [11]. The HIF hydroxylases belongs to the superfamily of 2-oxoglutarate (2-OG)-dependent oxygenases [12]. The identification of these enzymes provides potential targets that can activate HIF in the presence of oxygen. Prolyl hydroxylase (PHD) inhibitors have been the focus of recent studies on novel strategies to stabilize HIF. Dimethyloxalylglycine (DMOG), a cell penetrant analogue, inhibits PHD and activates HIF in cultured cells [8]. It also can be safely administered to experimental animals [13–15]. The activation of HIF was proved to be efficient in various kidney disease models including ischaemic or nephrototoxic acute kidney injury, chronic progressive glomerulonephritis and chronic kidney disease [15–20]. On
the other hand, there is also evidence that the activation of HIF signalling in renal epithelial cells is associated with the development of chronic kidney disease and that HIF may promote renal fibrosis [21,22]. These findings are in contrast to the renoprotective role of HIF. Therefore, it needs to be further determined whether HIF activation has a beneficial or an adverse effect on the chronic kidney disease model.

The remnant kidney is a disease model that mimics a situation of progression of chronic kidney disease in humans. In this model, initial glomerular hypertrophy and proteinuria are followed by glomerular sclerosis and tubulointerstitial fibrosis [23,24]. The aim was to evaluate the effect of HIF activation on the progression of renal injury in the rat remnant kidney. To investigate this, we observed the results of chronic continuous administration of DMOG or vehicle to the experimental animals from an early stage of renal mass reduction.

Materials and methods

Animals

Male Sprague-Dawley rats (Orient Bio Inc., Seongnam, Korea), approximately weighing 150 g, were used. This study was approved by, and conducted according to, the guidelines of the local animal ethics committee.

Experimental design

The rats \( n = 15 \) were randomly assigned to three groups. Two of these groups were assigned to undergo a subtotal nephrectomy and the other group to have a sham operation. After the right subcostal incision, the lower and upper thirds of the right kidney was exposed and separated from the adrenal gland under anaesthesia with ketamine and xylazine. The lower and upper thirds of the right kidney were removed. After 1 week, the left kidney was removed. The rats of the sham group underwent the same incision and manipulation of the left and the right kidneys without tissue destruction. Two weeks after the second surgical intervention, the rats of the subtotal nephrectomy groups were randomly assigned to receive either DMOG (Cayman, Ann Arbor, MI, USA) or vehicle, and the rats of sham group received vehicle alone. All rats were anesthetized with enflurane (Choongwae Pharma Corp, Seoul, Korea) and osmotic minipumps (model 2ML4; Alzet, Palo Alto, CA, USA) were subcutaneously implanted to deliver 15 mg/kg/day of DMOG or vehicle for 4 weeks. DMOG was dissolved to 50 mg/ml in phosphate-buffered saline (PBS). The vehicle consisted of PBS. After 4 weeks of DMOG or vehicle administration, the animals were anesthetized with ketamine and xylazine, blood samples were obtained and the kidneys were collected. One portion of the kidney was fixed in 10% phosphate-buffered formalin for morphologic and immunohistochemistry analyses, and the cortex and the medulla were dissected in another part. The remainder of the kidney was snap-frozen in liquid nitrogen and stored at \(-80\)°C for RNA and protein extraction.

Physiologic measurements

Before and after the administration of DMOG or vehicle, the rats were weighed and placed in metabolic cages, and urine was collected for 24 h. The urine volume was measured, and the protein concentration was determined by spectrophotometric assay [as modified by Lowry using bicinchoninic acid reagent (Pierce, Rockford, IL, USA)]. The urinary protein excretion was calculated in terms of milligrams of protein per 24 h. BUN and creatinine levels in the serum and urine were measured using an automatic analyser (ADVIA 1650, Siemens). The creatinine clearance was calculated using a standard formula and factored for body weight. Each rat’s systolic blood pressure (SBP) was monitored with a tail cuff sphygmomanometer (Panlab S.L., Barcelona, Spain). SBP was recorded as the mean value of three separate measurements that were obtained at each session. Determination of plasma malondialdehyde (MDA) concentration was measured spectrophotometrically using Oxiselect™ TBA assay kit (Cell Biolabs, San Diego, CA, USA).

Renal histologic and immunohistochemical analyses

Tissue for light microscopy and immunoperoxidase staining was fixed in a methyl Carnoy’s solution or formalin and embedded in paraffin. Three-micrometer sections were stained with periodic acid-Schiff (PAS) or Masson Trichrome (MT) staining. Direct immunoperoxidase staining of 3-μm sections was performed, as previously described [25], with the following specific antibodies: HIF-1α with rabbit polyclonal antibody (Novus Biologicals, Littletone, CO, USA); vascular endothelial growth factor (VEGF) with mouse monoclonal antibody (Abcam, Cambridge, UK); desmin with rabbit polyclonal antibody (Chemicon International, Temecula, CA, USA); podoplanin with mouse monoclonal antibody (AngioBio Co., Del Mar, CA, USA); monocyes/macrophages with ED-1 (Serotec, Oxford, UK); and osteopontin with rabbit anti-osteopontin antibody (Abcam).

Quantification of morphologic data

All analyses were performed in a blind manner. Segmental and complete glomerular sclerosis were analysed using a semiquantitative scoring system from 0 to 4 (0, no glomerulosclerosis; 1, <25% of glomerular area affected; 2, 25–50% affected; 3, 50–75% affected; 4, 75–100% affected). At least 30 glomeruli were evaluated under \( \times 400 \) magnification, and the results were averaged. The tubulointerstitial injury score was estimated based on the number of tubule dilatations, the distortion of the tubular basement membranes, and atrophy from 0 to 3 [0, none (<5%); 1, mild (5–15%); 2, moderate (25–50%); 3, severe (>50%)]. More than 10 consecutive fields were examined under \( \times 200 \) magnification and the results were averaged.

The stains for desmin were evaluated using a point counting method as published by Joles et al. [26]. Briefly, a grid composed of 400 dots was superimposed on glomeruli at \( \times 200 \) magnification, and the percentage of dots overlying the stained areas in 20 glomeruli was determined. The semiquantitative staining scores depended on the percentage of the glomerular edges that showed positive staining (0, 0–5% stained; 1, 5–25%; 2, 25–50%; 3, 50–75%; 4, >75%). Podoplanin staining was evaluated semiquantitatively on a scale from 0 to 3 [27] (0, normal intensity and pseudo-linear staining pattern; 1, loss of staining <25% of the glomerular surface or a granular staining pattern; 2, loss of staining involving 25–75% of the glomerular surface; and 3, loss of staining in >75% of the glomerular surface).

The mean numbers of infiltrating glomerular and interstitial macrophages (ED-1 positive cells) were calculated by averaging the total numbers of positive cells in at least 40 glomeruli and 30 sequentially selected, 0.25-mm² grids at \( \times 200 \) magnification.

Quantification of osteopontin immunostaining was done in the outer medullary region by counting the percentage of tubules with \( >50\% \) of cells staining positive for osteopontin. More than 10 consecutive fields were examined under \( \times 100 \) magnification, and the results were averaged.

Semiquantitative immunoblotting

Whole kidneys and the medulla were homogenized in a lysis buffer (250 mM sucrose, 10 mM triethanolamine, 1 μg/ml leupeptin, 0.1 mg/ml PMSF titrated to pH 7.6). Nuclear extracts were prepared from the renal medulla homogenates using a nuclear extraction kit (Sigma, St Louis, MO, USA). The total protein concentration was measured using a bicinchoninic acid protein assay reagent kit (Sigma) and was adjusted to 2 μg/ml with the isolation solution. The samples were then stabilized by adding 1 volume 5 × Laemmli sample buffer per 4 volume sample and heated to 60°C for 15 min. The samples were run on SDS-polyacrylamide gels (Bio-Rad Mini Protein III). Equal loading of protein was confirmed by staining identically loaded gels with Coomassie blue, as previously described [25]. The proteins were transferred to nitrocellulose membranes by electrophoresis. The following primary antibodies were used in this study: anti-HIF-1α (BD Bioscience, Franklin Lakes, NJ, USA), anti-catalase (Abcam) and anti-type IV collagen (Santa Cruz Biotech, Santa Cruz, CA, USA). Detection with peroxidase-conjugated secondary antibodies (Pierce no. 31458, Rockford, IL, USA) was followed by band visualization using an enhanced chemiluminescence substrate (ECL™ RPN 2106; Amersham Pharmacia Biotech, Buckinghamshire, UK) before exposure to X-ray film (Hyperfilm; Amersham Pharmacia Biotech, Buckinghamshire, UK). The
band densities were quantified by densitometry (GS-700 Imaging Densi
tometry, Bio-Rad, Hercules, CA, USA). To facilitate comparisons, the
densitometry values were normalized to a control, thereby defining the
mean for the control group as 100%.

Real-time PCR

Real-time PCR was performed to evaluate the mRNA expression of glu
cose transporter-1 (GLUT-1) and prolyl hydroxylase 3 (PHD3) in the
kidney of each group of rats. Total RNA was extracted from whole kidney
homogenates, and reverses transcribed with a Reverse Transcription Sys-
tem (Promega Corporation, Madison, WI, USA). Real-time PCR was per-
formed using the Applied Biosystems 7500 Fast Real-Time PCR System
(Applied Biosystems, Foster city, CA, USA), as described previously [25].
The primer/probe mixture was designed and provided by Applied Biosys-
tems. GLUT-1 mRNA, PHD3 mRNA and 18S rRNA were amplified in a
25 µL reaction volume with 10 µL of 2× TaqMan Universal Master Mix (Applied Biosystems) and 1 µL of 20× Assays-on-Demand (Rn00593670_m1 for GLUT-1, Rn00571341_m1 for PHD3 and
Hs99999901_s1 for 18S rRNA). Each sample was measured in tripli-
crate. 18S rRNA was used for normalization. The mRNA expressions are
presented as the relative values obtained from the rats of the sham group.

Statistical analysis

All of the data are presented as means ± SEM. The statistical analyses were
performed using SPSS (version 15.0 for Windows; SPSS Inc., Chicago,
IL, USA). Data were analysed using Student’s t-test for comparisons.
Statistical significance is indicated by *P < 0.05.

Results

Physiological findings

At the end of the study, rats that received a nephrectomy had a reduced body weight, higher SBP and lower haemoglobin level than the sham-operated rats did (Table 1). The BUN level remained increased after renal mass reduction, but was not significantly influenced by the treatment with DMOG. Rats that received DMOG experienced no further increase of serum creatinine after the nephrectomy, whereas vehicle-
treated rats showed a significantly increased serum creati
nine level with respect to baseline value. Furthermore, the change of serum creatinine levels before and after vehicle/
DMOG administration was greatest in vehicle-treated rats with a nephrectomy as compared with DMOG-treated rats with a nephrectomy or sham-operated rats. However, the creatinine clearance did not show any statistical difference with respect to the DMOG treatment. The base
line proteinuria amount was equivalent in both groups that underwent a nephrectomy. While vehicle-treated rats excreted twice the amount of urinary protein after 4 weeks, the amount of proteinuria did not change for the DMOG-
treated rats.

Histologic analysis

Figure 1 shows a representative PAS and MT staining of the kidney from each group of animal. In the nephrectomized rats that received DMOG, the severity of glomeruloscle
rosis was significantly reduced (score: 1.33 ± 0.1 versus 1.95 ± 0.12, DMOG versus vehicle, *P < 0.05). The degree of tubulointerstitial injury was also significantly reduced in the nephrectomized rats that received DMOG compared with the nephrectomized rats that received vehicle (score:

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Table 1. Physiological data of experimental animals

<table>
<thead>
<tr>
<th></th>
<th>Sham vehicle (n = 5)</th>
<th>Vehicle (n = 5)</th>
<th>DMOG (n = 5)</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>501 ± 5</td>
<td>383 ± 21*</td>
<td>385 ± 16*</td>
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<td>SBP (mmHg)</td>
<td>135 ± 3</td>
<td>177 ± 6*</td>
<td>156 ± 11*</td>
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<tr>
<td>Haemoglobin (g/dl)</td>
<td>13.9 ± 0.4</td>
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<td>11.6 ± 1.0*</td>
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<tr>
<td>BUN (mg/dl)</td>
<td>12.0 ± 1.3</td>
<td>38.0 ± 2.3*</td>
<td>35.4 ± 3.6*</td>
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<tr>
<td>Final ΔBUN</td>
<td>11.2 ± 0.6</td>
<td>46.4 ± 4.0*</td>
<td>42.2 ± 4.3*</td>
</tr>
<tr>
<td>Cr (mg/dl)</td>
<td>−0.8 ± 1.4</td>
<td>8.4 ± 1.9*</td>
<td>6.8 ± 3.3*</td>
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<tr>
<td>Clcr (ml/min/100 g)</td>
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<td>0.70 ± 0.08*</td>
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<td>Baseline Final</td>
<td>0.34 ± 0.02</td>
<td>1.00 ± 0.10*</td>
<td>0.76 ± 0.07*</td>
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<tr>
<td>ΔCr</td>
<td>−0.03 ± 0.08</td>
<td>0.26 ± 0.05*</td>
<td>0.06 ± 0.07</td>
</tr>
<tr>
<td>Urine protein (mg/day)</td>
<td>0.58 ± 0.07</td>
<td>0.29 ± 0.03*</td>
<td>0.33 ± 0.07*</td>
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<td>Baseline Final</td>
<td>0.48 ± 0.15</td>
<td>0.24 ± 0.05</td>
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<td>ΔClcr</td>
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<td>−0.05 ± 0.03</td>
<td>−0.02 ± 0.05</td>
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<td>Proteinuria</td>
<td>1.6 ± 0.4</td>
<td>36.7 ± 12.2*</td>
<td>36.3 ± 8.8*</td>
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<td>Δproteinuria</td>
<td>2.2 ± 0.8</td>
<td>78.1 ± 17.7*</td>
<td>43.4 ± 5.9*</td>
</tr>
</tbody>
</table>

*P < 0.05 versus sham; #P < 0.05 versus baseline value.
DMOG, dimethyloxalylglycine; SBP, systolic blood pressure; Cr, creati
nine; Clcr, creatinine clearance normalized per body weight.

1.45 ± 0.14 versus 2.45 ± 0.11, DMOG versus vehicle, (P < 0.05).

HIF activation

We examined whether DMOG treatment activated HIF-1α. A dose and route of administration of DMOG was de
termined by the pilot experiment (data not shown). The up-regulation of intranuclear HIF-1α protein by DMOG in
fusion was demonstrated by Western blotting. The specific band corresponding to HIF-1α disappeared in the vehicle-
treated nephrectomized rats, but it was detected in the DMOG-treated nephrectomized rats (Figure 2A). Likewise, immuno
histochemical studies showed that the expression of HIF-1α was restored by DMOG treatment (Figure 2B–D).
Next, we investigated the expression of GLUT-1 and PHD3 to determine whether DMOG treatment activated the HIF-
regulated gene (Figure 2E and F). Although mRNA lev
els of GLUT-1 (0.19 ± 0.06 fold versus sham) and PHD3 (0.09 ± 0.04 fold versus sham) were significantly decreased after the nephrectomy, the DMOG treatment increased and partially restored them to the baseline levels (GLUT-1, 0.41 ± 0.2 fold; PHD3, 0.3 ± 0.26 fold). The immuno
staining of VEGF, another target gene of HIF, was markedly decreased by nephrectomy, but it was restored by DMOG

treatment (Figure 2G–I).

Podocyte injury

Immunohistochemical studies showed up-regulation of desmin and down-regulation of podoplanin in the rats that
received a nephrectomy (Figure 3). In the kidneys of sham-operated rats, desmin staining was barely observed in the glomeruli. In contrast, podoplanin protein expression was normal. In the rats that received a nephrectomy and vehicle treatment, conspicuously enhanced desmin staining and loss of podoplanin staining were observed whereas DMOG treatment resulted in a decrease in desmin expression and an increase in podoplanin expression. A semiquantitative analysis confirmed a mitigation of desmin expression (score: 1.6 ± 0.09 versus 2.58 ± 0.08) and a restoration of podoplanin expression (score: 2.14 ± 0.09 versus 1.67 ± 0.11) by DMOG.

**Oxidative stress**

To detect oxidative stress, we measured plasma MDA levels in the experimental animals. Despite the statistical insignificance, plasma MDA level showed a tendency to increase after nephrectomy and to decrease by DMOG treatment (55.4 ± 13.4 µM versus 76.9 ± 11.7 µM versus 52.0 ± 21.8 µM, sham versus vehicle versus DMOG). Vehicle-treated nephrectomized rats exhibited a significant reduction in the abundance of catalase, an antioxidant, when compared with the sham-operated rats (Figure 4). The DMOG treatment significantly increased the abundance of catalase. The normalized band densities for the DMOG- and vehicle-treated nephrectomized rats were 81 ± 2% and 68 ± 3%, respectively.

**Inflammation**

Subtotal nephrectomy was associated with macrophage infiltration in the tubulointerstitium as determined by an increase in ED-1-positive cells (Figure 5). By counting the absolute number of ED-1-positive cells, we observed a marked increase in macrophage infiltration after nephrectomy and a significant reduction in response to DMOG treatment. The mean ED-1 counting score was 26.2 ± 1.8 in DMOG-treated rats and 43.6 ± 2.1 in vehicle-treated rats.

**Fibrosis**

Renal fibrosis, measured via semiquantitative immunoblotting of type IV collagen from whole kidney homogenates, was dramatically attenuated in the DMOG-treated rats (140 ± 14 versus 908 ± 320%, DMOG versus vehicle; Figure 6). Osteopontin expression by tubules, which is correlated with the development of fibrosis, was also significantly reduced by DMOG treatment (Figure 7A–C). Quantification of tubular osteopontin staining indicated that the percentage of positive tubules was significantly decreased from 64 ± 8.3% to 19.2 ± 6.2% by DMOG (Figure 7D).

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**Fig. 1.** DMOG improves renal histology. Representative pictures of PAS-stained (A–C) and MT-stained (D–F) renal tissues. (A and D) Sham operation and vehicle treatment (B and E) nephrectomy and vehicle treatment and (C and F) nephrectomy and DMOG treatment. Histological evaluation of glomerular sclerosis (G) and tubulointerstitial injury (H). *Significantly different with respect to sham-operated rats; #Significantly different with respect to vehicle treatment; \(^{*}P < 0.05\). Magnification ×400 (A–C) and ×200 (D–F).
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Fig. 2. Activation of hypoxia-inducible factor (HIF). Western blotting of HIF-1α in the nuclear extracts of renal medullary homogenates from experimental animals (A). Immunohistochemical stains for HIF-1α of a representative kidney of rats with a sham operation and vehicle treatment (B), nephrectomy and vehicle treatment (C) or nephrectomy and DMOG treatment (D). GLUT-1 or PHD3 mRNA expression determined by RT real-time PCR using total renal RNA from rats of each group (E, F). The baseline expression levels of GLUT-1 or PHD3 mRNA in the sham-operated rats were arbitrarily set at 1. *Significantly different with respect to sham-operated rats; #Significantly different with respect to vehicle treatment; **p < 0.05. Immunohistochemical stains for VEGF of a representative kidney of rats with a sham operation and vehicle treatment (G), nephrectomy and vehicle treatment (H), or nephrectomy and DMOG treatment (I). Magnification ×40.

Discussion

This study demonstrated that the activation of HIF produced a renoprotective effect by preventing podocyte injury in the early phases of renal mass reduction. To our knowledge, this is the first study to demonstrate the effects of DMOG, a HIF activator that inhibits PHD, on podocyte injury in the remnant kidney model. DMOG prevented the further increase of proteinuria and attenuated glomerular as well as tubulointerstitial injury in this model, which was associated with anti-oxidative, anti-inflammatory and anti-fibrotic effects.

We started to administer DMOG 2 weeks after the rats underwent a nephrectomy, before significant anatomical and functional disturbances had occurred, and continued the treatment for 4 weeks. Despite the pronounced changes in histology score between the DMOG-treated and vehicle-treated nephrectomized rats, no significant differences were observed with regard to proteinuria or serum creatinine levels. However, the progression of proteinuria or elevation of the serum creatinine levels was halted by DMOG treatment. We speculate that these physiological results are due to the timing of the observation. If we had ended this study >8 weeks after nephrectomy, we would have observed inter-group differences in the physiological data as reported by other studies [20]. However, we focused the effect of HIF activation starting at an early stage of renal mass reduction in this study because glomerular hypertrophy occurred as early as 4 weeks after nephrectomy [23, 28–30].
Fig. 3. DMOG attenuates podocyte injury. Immunohistochemical stains for desmin and podoplanin obtained from a representative kidney of rats with a sham operation and vehicle treatment (A, D), nephrectomy and vehicle treatment (B, E) or nephrectomy and DMOG treatment (C, F). Desmin counting score (G). Score of podoplanin loss (H). *Significantly different with respect to sham-operated rats; #Significantly different with respect to vehicle treatment; **P < 0.05. Magnification ×200.

Fig. 4. Effects of DMOG on the abundance of catalase. Representative Western blot and group data depicting catalase protein abundance in the kidneys of rats of each group. Parallel Coomassie blue-stained SDS-polyacrylamide gels demonstrated a uniform loading among all samples (not shown), which rules out the possibility that the increase in band density could be due to differences in loading. *Significantly different with respect to sham-operated rats; #Significantly different with respect to vehicle treatment; **P < 0.05.

There have been studies that reported a renoprotective effect of vascular endothelial growth factor (VEGF) or cobalt through the improvement of angiogenesis in the remnant kidney model [20,31]. VEGF is one of the target genes of HIF and cobalt is a HIF activator. However, podocyte injury and the degree of glomerulosclerosis were unaffected by those treatments. In our study, glomerular and tubulointerstitial injuries were markedly improved by DMOG treatment. The results of the renal histology obtained in our study could be attributed to an earlier start of treatment compared with the above studies.

Even though we did not try to detect tissue hypoxia, hypoxia has been reported to persist from early to advanced stages of the remnant kidney [20,32]. However, the expression of HIF and its target genes were decreased rather than increased despite persistent hypoxia after renal mass reduction in our study. We suppose that long-term hypoxia may decrease HIF protein, which was increased right after the nephrectomy. We observed that a chronic administration of DMOG to rats led to successful activation of HIF and an induction of its downstream genes in the remnant kidney.

In the animal model of renal mass reduction, major structural alterations in the glomeruli begin with podocytes. As podocytes have only a limited potential for cell replication [33–36], they adapt to mechanical stress via glomerular hypertrophy and glomerular capillary hypertension that result from renal ablation, by changing their structures [23]. Finally, podocytes fail to adapt to the enlargement of the capillary surface and detach from the glomerular basement membrane (GBM). The denuded GBM proceeds to sclerosis, and focal segmental glomerulosclerosis develops...
Fig. 5. DMOG reduces the number of infiltrating macrophages. Immunohistochemical stains for ED-1 of a representative kidney of rats with a sham operation and vehicle treatment (A), nephrectomy and vehicle treatment (B), or nephrectomy and DMOG treatment (C). ED-1 counting score (D). *Significantly different with respect to sham-operated rats; #Significantly different with respect to vehicle treatment; \( ^{*}P < 0.05 \). Magnification \( \times200 \).

Fig. 6. Effects of DMOG on the abundance of type IV collagen. Representative Western blot and group data depicting type IV collagen protein abundance in the kidneys of rats of each group. Parallel Coomassie blue-stained SDS-polyacrylamide gels demonstrated a uniform loading among all samples (not shown), which rules out the possibility that the increase in band density could be due to differences in loading. *Significantly different with respect to sham-operated rats; \(^{*}\)Significantly different with respect to vehicle treatment; \(^{**}\)\( P < 0.05 \).

Further in vitro experiments using podocytes need to be performed to determine whether HIF is directly involved in the protection of podocyte injury as well as to elucidate the underlying mechanism of their protection.

Chronic kidney disease is associated with oxidative stress [40–43], the precise mechanism of which is yet to be elucidated. In the animal model of renal mass reduction, an up-regulation of NAD(P)H oxidase and an impairment of the antioxidant defence system have been suggested as the pathogenesis of oxidative stress in chronic kidney disease [44–46]. Oxidative stress can occur either as a result of an increased reactive oxygen species (ROS) generation, a depressed antioxidant system or both. The primary ROS produced in the organisms is superoxide, which is a cytotoxic agent [47]. Plasma level of MDA, marker of lipid peroxidation, showed a tendency to change by nephrectomy or DMOG treatment in this study. Catalase is a peroxidase enzyme that is one of the major antioxidant defence systems, and its deficiency might cause oxidative stress. In our study, an abundance of the protein catalase was significantly reduced by nephrectomy, in agreement with previous studies [45,48]. However, HIF activation attenuated this down-regulation of catalase. Therefore, HIF activation can protect a remnant kidney from oxidative stress.

The renoprotective effect of DMOG was also associated with reduced inflammation and fibrosis. Because proteinuria itself has a strong association with interstitial macrophage infiltration [49], amelioration of proteinuria by DMOG might inhibit the infiltration of macrophages in our study. Aside from HIF activation, DMOG also induces NF\( \kappa \)B activity resulting in the reduction of inflammation.
in a model of colitis [50]. Therefore, there is a possibility that activation of NFκb signalling by DMOG reduced inflammation in this study. Although the precise mechanism by which inflammatory cells cause fibrosis is uncertain, most data suggest that macrophages promote renal fibrosis [51,52]. Therefore, the decrease of inflammatory cells caused by DMOG treatment might subsequently attenuate fibrosis in the remnant kidney of this study.

In summary, activation of HIF by DMOG treatment reduced the increase of proteinuria and structural damage by preventing podocyte injury in a model of renal mass reduction. This renoprotection was accompanied by a reduction of oxidative stress, inflammation and fibrosis. Our observations confirmed that HIF PHD inhibitors seemed to be safely used in humans [53], pharmacological activation of HIF would provide a good therapeutic strategy for treating chronic kidney disease.

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Conflict of interest statement. None declared.

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