Use of a hanging-weight system for isolated renal artery occlusion during ischemic preconditioning in mice

Almut Grenz,1* Tobias Eckle,2* Hua Zhang,1 Dan Yang Huang,1 Manfred Wehrmann,2 Christoph Köhle,1 Klaus Unertl,2 Hartmut Osswald,1 and Holger K. Eltzschig2

Departments of 1Pharmacology and Toxicology, 2Anesthesiology and Intensive Care Medicine, and 3Pathology, Tübingen University Hospital, Tübingen, Germany

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Grenz A, Eckle T, Zhang H, Huang DY, Wehrmann M, Köhle C, Unertl K, Osswald H, Eltzschig HK. Use of a hanging-weight system for isolated renal artery occlusion during ischemic preconditioning in mice. Am J Physiol Renal Physiol 292: F475–F485, 2007. First published August 15, 2006; doi:10.1152/ajprenal.00275.2006.—Renal failure from ischemia contributes to morbidity and mortality. Ischemic preconditioning (IP) represents a powerful strategy for kidney protection, and recent advances in transgenic mice may help elucidate its molecular mechanisms. However, murine IP is technically challenging and experimental details significantly influence results. Thus we developed a novel model for renal IP using a hanging-weight system for isolated renal artery occlusion. In contrast to previous models, this technique eliminates the need for clamping the vascular pedicle (artery/vein). In fact, assessment of renal injury after different time periods of ischemia (10–60 min) revealed highly reproducible increases in plasma creatinine and potassium levels, while creatinine clearance, urinary flow and potassium/sodium excretion were significantly attenuated. Using different numbers of IP cycles, we found maximal protection with four cycles of 4 min of ischemia-reperfusion. In contrast, no significant renal protection was observed with IP of the vascular pedicle. To assess transcriptional responses in this model, we isolated RNA from preconditioned kidneys and found time-dependent induction of erythropoietin mRNA and plasma levels with IP. Taken together, this model provides highly reproducible renal injury and protection by IP, thus minimizing variability associated with previous techniques based on clamping of the renal pedicle. Further studies on renal ischemia/IP in mice may consider this technique.

targeted gene deletion; murine; acute renal failure; ischemia; reperfusion; kidney

COMPARSED WITH OTHER ORGANS, the kidneys are particularly sensitive to ischemia. In fact, only relatively short time periods of limited supply with blood and/or oxygen can precipitate acute renal failure. Therefore, it is not surprising that ischemic injury to the kidneys significantly affects morbidity and mortality of patients in many clinical settings, including renal transplantation (6), intensive care medicine (1), or of patients undergoing major vascular or open heart surgery (7, 24). Therefore, studies on renal protection from ischemia are currently areas of intense investigation. A powerful strategy to improve ischemic tolerance of the kidneys is ischemic preconditioning (IP). Already since the 1980s, studies have demonstrated pretreatment with ischemia transiently lowers renal resistance to a second ischemic injury (31). Since then, multiple attempts have been undertaken to identify molecular mechanisms involved in renal protection by IP. Despite these efforts, molecular mechanisms involved in renal protection by IP remain largely unknown. In addition, none of these concepts could be translated into a clinical setting, resulting in reduction of morbidity and mortality from acute renal failure as profound as would be expected from the experimental results. However, the recent availability of transgenic mice offers hope that molecular mechanisms of renal protection by IP will soon be unraveled. Moreover, the use of “floxed” (12, 22) or chimeric (30) mice may yield additional insight into the contribution of individual tissues or cell lines (e.g., renal tubular cells, endothelia or myeloid cell lines). For example, recent studies using chimeric mice have suggested that attenuation of ischemia-reperfusion injury of the kidneys can be attenuated by activation of adenosine receptors located on T lymphocytes (9). This kind of information is very important for pharmacological approaches, as drug design clearly depends on the tissue of interest.

Previous studies have already demonstrated that renal IP can be performed in mice (3, 26, 29). However, these studies differ in their experimental approach, particularly with regard to details of intermittent occlusion of blood flow to the kidneys. One approach is clamping of the renal vascular pedicle (artery, vein) (3). Pediculocclusion is usually achieved by clamping the renal vein and artery, thus bearing the potential problem of venous congestion or outflow obstruction during ischemia. Moreover, this technique may be associated with tissue trauma during IP by removing and replacing the surgical clamp. Furthermore, exact reproducible durations of IP intervals with clamps are difficult to perform by removing and replacing of the surgical clamps. Based on these potential problems associated with pedicle occlusion by a clamp, we adopted a previously published model of cardiac IP (10) using a hanging-weight system for intermittent and selective occlusion of the renal artery (Fig. 1). In fact, isolated and intermittent renal artery occlusion could be achieved without need for a surgical clamp. Moreover, reperfusion is obtained by simply supporting the hanging weights. In fact, we hypothesized that this model would yield highly reproducible parameters of kidney injury by ischemia in conjunction with renal protection by IP, as it allows for immediate and reliable renal artery occlusion/reperfusion in combination with virtually no tissue trauma or renal

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congestion as may be seen with clamping of the pedicle. In fact, we tested this model systematically, including variation of ischemia time and numbers of preconditioning cycles. In fact, we found that this technique is more reliable for renal protection by IP compared with clamping of the pedicle. Therefore, this technique may be useful in further studies of renal protection from ischemia by IP in murine models.

MATERIALS AND METHODS

Anesthesia and surgery. All experimental protocols were conducted in accordance with the German law on animal protection and approved by the Regierungspräsidium in Tübingen. C57BL/6 mice were age (4–6 wk old) and gender matched. The animals were kept on a regular 12:12-h dark-light cycle with free access to standard chow (Altromin 1320, Altromin, Lage, Germany) and tap water.

Renal IP experiments can either be performed by simultaneously applying ischemia and reperfusion to both kidneys or after single nephrectomy in combination with IP treatment of the remaining kidney. As bilateral IP treatment of both kidneys is associated with increased technical difficulties, a more complex experimental setting, and longer surgeries, we performed a right nephrectomy followed by ischemic treatment of the remaining left kidney. For this purpose, animals were anesthetized with pentobarbital sodium (70 mg/kg) and placed on a temperature-controlled heating table (to maintain body temperature at 37°C; RT, Effenberg, Munich, Germany) in a left lateral decubitus position, and the left kidney was carefully removed from the connective tissue and turned with its ventral side down into a lucite cup. D: the renal artery (renal art.) which runs on top of the renal vein was identified close to its take-off from the aorta. After the vessel was dissected from adjacent tissues (between its take-off from the abdominal aorta and its bifurcation at the renal pelvis), an 8/0 nylon suture was fitted under the artery. E: arterial blood flow to the kidney was interrupted by applying small weights to the suture, while blood flow through the renal vein remained unobstructed. Interruption of blood flow through the renal artery was confirmed by an immediate change in the color of the kidney from red (renal artery open; F) to white (renal artery occluded; G).
using 6/0 nylon sutures. The surgical wound was closed as described above. At the end of surgery, mice received 0.3 ml normal saline (ip), recovered for 2 h under a heating lamp, and then were placed into metabolic cages (Tecniplast Deutschland, Hohenpeissenberg, Germany) for determination of renal functional parameters. In subsets of experiments, blood pressure and heart rate were recorded throughout the surgical procedure. As described previously (10), blood pressure measurements were obtained continuously via a carotid artery catheter. In brief, the carotid artery was exposed via blunt dissection of the paratracheal muscles. Following further exposure and careful avoidance of tissue trauma (particular of the vagal nerve), a catheter was inserted into the vessel using two sutures and a small clamp. Blood pressure was measured with a Statham element (WK 280, WKK, Kaltbrunn, Switzerland).

Technique of renal artery occlusion. Operations were performed under an upright dissecting microscope (Leica, MZ95, Bensheim, Germany). Following exposure of the left kidney and placement into a lucite cup, the renal artery was easily identified as it runs on top of the renal vein. The vessel was dissected from adjacent tissues, close to its take-off from the abdominal aorta. Then, an 8/0 nylon suture (Ethicon, Norderstedt, Germany) was placed around the artery (Fig. 1A). This technique allows interruption of only the arterial blood flow to the kidney without compression of the renal vein, compared with clamping of the renal pedicle (25). The suture was placed over a small pole, and a 1-g weight was attached to each end (Fig. 1B). While the weights were unsupported, the renal artery was immediately occluded (Fig. 1, E and G). In contrast, when the weights were supported, blood flow was immediately restored to the organ (Fig. 1, D and F). Successful occlusion was confirmed by a change in color from red to white (Fig. 1, F and G). During reperfusion, the color change immediately disappeared when the hanging weights were supported and the kidney was reperfused. In additional experiments, we performed IP of the kidney by applying a surgical clamp to the renal pedicle (renal artery and vein). In this experimental group, ischemia following IP was achieved in a similarly fashion (by clamping of the renal pedicle, artery, and vein). In contrast to using the hanging-weight system for isolated renal artery occlusion, we did not observe an immediate and complete change in color with clamping of the renal pedicle. In fact,
the kidney turned dark blue after clamping of the pedicle, most likely due
to venous congestion. All animals survived the surgical procedure, and no
complications were observed with renal artery occlusion using the hang-
ing-weight system or in controls using pedicle clamping.

Preconditioning protocols. As a first step, the influence of different
ischemia times (10, 20, 30, 45, and 60 min) on kidney function was
investigated. Then, renal protection by IP was assessed in this model
and different IP regimens with different numbers of ischemia-reperfusion
cycles were tested.

Assessment of renal function after ischemia-reperfusion injury with
and without IP. Mice were placed in metabolic cages (Tecniplast
Deutschland) 2 h after the experimental procedure. Renal function
was assessed by measuring plasma and urine creatinine 24 h after
renal ischemia using a commercially available colorimetric method
according to the manufacturer’s protocol (LT-SYS, Labor + Technik,
Berlin, Germany). Plasma and urine concentrations of Na⁺ and K⁺
were determined with a flame-emission photometer (ELEX 6361,
Eppendorf, Hamburg, Germany). Renal excretory and hemodynamic
values were calculated using standard formulas. Mice were killed after
24 h of observation in the metabolic cage, and plasma samples were
obtained. In addition, the kidneys were harvested and stored at −80°C
until further analysis.

Histological evaluation. For histological assessment, kidney tissues
were fixated in 4.5% buffered formalin, dehydrated, and embedded in
paraffin. Sections (3 μm) were stained with hematoxylin, eosin, and
periodic acid-Schiff. Examination and scoring of the whole section of
each kidney were carried out by a board-certified renal pathologist
who was blinded to the experimental group. A grading scale of 0–4,
as outlined by Jablonski et al. (16), was used for the histopathological
assessment of proximal tubular damage by ischemia and reperfusion
in kidneys with or without IP. Four animals were used in each
condition, and three representative sections from each kidney were
scored.

Immunohistochemistry. Granulocytic infiltrates were stained by
histochemistry using chloracetate esterase (14). Examination and
scoring for neutrophil infiltration of sections from each kidney were
carried out by a board-certified renal pathologist who was blinded to
the treatment group. In short, 0–4 points were given depending on the
degree of infiltration: grade 0, normal renal tissue; grade 1, mild
localized infiltration; grade 2, moderate infiltration in different areas;
grade 3, severe infiltration (<50%); and grade 4, severe diffuse
infiltration of >50% of renal tissue. Six animals were used in each
condition, and three representative sections from each kidney were
scored.

Myeloperoxidase activity. To evaluate neutrophil and macrophage
infiltration, we modified previously described methods (13, 21). In
short, snap-frozen kidney samples were homogenized in ice-cold 50
mM potassium phosphate buffer, pH 7.4, and then centrifuged at

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**Fig. 3.** A: schematic illustration of the experimental pro-
tocol for renal IP. B: to optimize renal protection by IP,
the effect of different numbers of IP cycles on plasma
creatinine clearance was assessed. For this purpose, mice
underwent right nephrectomy and IP was performed using
the hanging-weight system for isolated renal artery occlu-
sion with indicated numbers of IP cycles before 30 min of
ischemia. One IP cycle consisted of 4-min ischemia fol-
lowed by 4 min of reperfusion. Following the surgery,
mice were placed in metabolic cages over 24 h and plasma
creatinine levels were measured. Results are means ± SD
derived from 6–8 mice/condition. P values indicate dif-
ferences between experimental groups and mice without
IP treatment (0 cycles).
20,000 g for 15 min at 4°C. The supernatant was discarded, and the pellet was resuspended in 50 mM potassium phosphate buffer, pH 7.4, containing 0.5% (wt/vol) hexadecyltrimethylammonium bromide and sonicated for 30 s. Homogenates were centrifuged at 20,000 g for 15 min at 4°C. Five microliters of the supernatant were added to 195 μl of reaction buffer (50 mM potassium phosphate buffer, pH 6.0, 0.68 mM O-dianisidine, 0.0005% hydrogen peroxide). The change in absorbance was measured spectrophotometrically (Victor 3V, PerkinElmer) at 450 nm over 2 min. Myeloperoxidase activity was expressed as a change in optical density (OD) per minute per milligram of protein which was detected by protein Lowry assay.

**Real-time PCR.** Total RNA was isolated from renal tissue using a total RNA isolation NucleoSpin RNA II Kit according to the manufacturer’s instructions (Macherey & Nagel, Düren, Germany). For this purpose, frozen tissue was homogenized in the presence of RA1 lysis buffer (Micra D8 homogenizer, ART-Labortechnik, Müllheim, Germany) and after filtration lysates were loaded on NucleoSpin RNA II columns, followed by desalting and DNase I digestion (Macherey & Nagel).

cDNA synthesis was performed using reverse transcription according to the manufacturer’s instructions (i-script Kit, Bio-Rad Laboratories, Münich, Germany). RNA was washed and the concentration was quantified. The primer sets for RT-PCR contained 1 μM sense and 1 μM antisense with SYBR Green I (Molecular Probes). Primer sequences for EPO were the following: EPO (RefSeq Accession no. NM_007942) sense: 5’-AATGGAGGTTGAAAGACAGG-3’; antisense: 5’-ACCGAAGCGTAAGTGA-3’; and β-actin: sense: 5’-TCTGGCACCACACTTCTACA-3’, antisense: 5’-GGGTTGGTGAAAGGTCTCAAAC-3’. β-Actin served as an internal control. The DNA was amplified using increasing numbers of cycles of 94°C for 1 min, 62°C for 0.5 min, and 72°C for 1 min.

**Erythropoietin plasma levels.** Plasma EPO concentrations were determined by ELISA with a commercially available kit, following the manufacturer’s instructions (Medac).

**Data analysis.** Renal injury score data are given as median (range); all other data are presented as means ± SD from 5–10 animals/condition. We performed statistical analysis using Student’s t-test (2 sided, α < 0.05) or analysis of variance to determine group differences. Renal injury was analyzed with a Kruskal-Wallis rank test.

**RESULTS**

**Renal injury after ischemia.** As a first step, we tested the influence of different ischemia times on renal injury using the hanging-weight system for isolated renal artery occlusion. In fact, identification of an ischemia time resulting in a medium range of renal damage is critical for the study of renal protection by IP, as it allows identification of changes in both directions, e.g., smaller degree of injury with renal IP or larger degree of injury with experimental therapeutics or a specific gene deletion. In addition, it is important to use an ischemia time that is associated with some degree of remaining renal function, as studies of clearance, etc. would not be possible in anuric animals. As shown in Fig. 2A, ischemia times from 0 to 60 min followed by 24 h of reperfusion were associated with increased plasma creatinine and decreased creatinine clearance (Fig. 2A). In fact, over the examined time range (0–60 min), renal ischemia time closely correlated with plasma creatinine levels ($R^2 = 0.98$). Similarly, urinary potassium excretion decreased while plasma potassium concentrations increased with longer ischemia times (Fig. 2B). In addition, urinary flow rate (Fig. 2C) and urinary sodium excretion (Fig. 2D) were attenuated with renal arterial ischemia. Taken together, these results provide feasibility of using isolated renal artery occlusion by a hanging-weight system for inducing highly reproducible and time-dose-dependent renal injury. Since we observed a “medium” degree of renal injury with 30 min of ischemia time, all further studies were performed using 30 min of selective renal artery occlusion followed by 24 h of reperfusion.

**Renal protection by IP.** After having demonstrated reproducible renal injury with ischemia and reperfusion, we next used the hanging-weight occlusion system in experiments of
renal protection by IP. For this purpose, we used different cycle numbers of IP, each consistent with 4 min of ischemia and 4 min of reperfusion, followed by 30 min of ischemia time and 24 h of reperfusion (Fig. 3A). Under these conditions, one or two cycles of IP were not associated with a significant attenuation of the plasma creatinine concentration compared with unpreconditioned animals (Fig. 3B). In contrast, three, four, or five cycles of IP were associated with renal protection. In fact, we found maximal protection with four cycles, resulting in a 2.6-fold reduction of plasma creatinine from 2.9 ± 0.4 to 1.1 ± 0.5 mg/ml (P < 0.0001, Figs. 3B and 4A). This robust improvement in kidney function by IP was confirmed in other renal function tests, including a reduction of plasma potassium levels (Fig. 4B) and improved creatinine clearance (Fig. 4C), urinary flow rate (Fig. 4D), and urinary excretion of sodium (Fig. 4E) and potassium (Fig. 4F). Moreover, histological signs of ischemic injury were attenuated by IP (Fig. 5). As shown in Fig. 5A, 30 min of ischemia resulted in severe acute tubular necrosis, as can be seen by the loss of tubular cell nuclei in the cortex and outer medullary stripe with extensive disappearance of the proximal tubular brush border. Large numbers of casts containing brush border blebs were observed in the outer medulla. Hyaline cast formation and intraluminal necrotic cellular debris could be seen in the cortex as well as in the outer medulla. In contrast, mice with IP treatment before ischemia (Fig. 5B) showed only mild to moderate histological signs of acute tubular necrosis, as can be seen by intact tubular cell morphology. The proximal tubular brush border damage was sporadic and quantitatively mild. In addition, a moderate numbers of hyaline casts were apparent in the IP-treated group. In fact, semiquantitative histological analysis demonstrated a reduction in the Jablonski index from grade 3 (range 3–4) without IP to grade 2 (range 1–3, Fig. 5C, P < 0.01) with IP. Similarly, signs of acute renal inflammation were attenuated after IP, as demonstrated by cytochemical staining of granulocytes with chloracetate esterase (Fig. 6, A and B). Renal tissue following 30 min of ischemia without IP revealed a significant increase in granulocyte infiltration, preferentially localized in peritubular areas compared with the IP-treated group. Thus quantitative analysis showed a reduction in granulocyte infiltration from grade 3.5 (range 3–4) without IP to grade 2 (range 1–3, Fig. 6C, P = 0.001). Furthermore, mice subjected to IP before 30 min of ischemia showed less tissue myeloperoxidase activity (Fig. 6D, P = 0.01).

**Comparison of isolated renal arterial occlusion with clamping of the pedicle.** As a next step, we compared renal protection by IP via isolated renal artery occlusion using the hanging-weight system with conventional clamping of the whole renal pedicle (artery and vein). As shown in Fig. 7, IP (4 cycles) of isolated renal artery occlusion using the hanging-weight system resulted in a robust reduction of renal injury by ischemia (30 min). In contrast, using a similar IP protocol with clamping

![Fig. 5. Histological signs of renal injury by ischemia are attenuated following IP. A and B: for investigation of histological aspects of renal protection by IP, mice underwent right nephrectomy followed by IP of the remaining kidney using the hanging-weight system for isolated renal artery occlusion (4 IP cycles with 4-min ischemia and 4 min of reperfusion before 30 min of ischemia). Following the surgery, mice were recovered over 24 h. Representative sections are displayed (×200 and ×400, following hematoxylin and eosin staining). C: quantification of ischemic injury with the Jablonski scale (see MATERIALS AND METHODS). Results are means ± SD derived from 6–7 mice/condition. P values indicate differences between −IP and +IP mice.](http://ajprenal.physiology.org/)
of the whole pedicle before a 30-min ischemia time was not associated with a statistically significant improvement in kidney function. In fact, no significant reduction of plasma creatinine or potassium nor an improvement of the creatinine clearance could be demonstrated with IP (Fig. 7, A–C). Similarly, urinary flow rate and urinary potassium and sodium excretion were not improved when IP was performed via clamping of the whole pedicle (Fig. 7, D–F). These results confirm our initial hypothesis that IP via isolated renal artery occlusion provides a more profound and reliable protection of the kidney from ischemia than clamping of the whole pedicle.

Modulation of gene expression by renal IP. As a last step, we measured gene regulation by IP in this model. For this purpose, we performed IP as shown in Fig. 8A and removed the kidney at indicated time points for mRNA isolation and transcriptional analysis. To demonstrate gene regulation by IP in this model, we investigated erythropoietin transcription at different time points after IP treatment. In fact, erythropoietin has been shown to be induced by ischemia or hypoxia (27) and plays an important role in renal protection from ischemic injury (28). Similar to these studies, we found induction of erythropoietin mRNA by renal ischemia in the presented model (Fig. 8B). In addition, we found a close correlation of erythropoietin mRNA with erythropoietin plasma levels (Fig. 8C), suggesting that induction of erythropoietin is not only limited to increases in mRNA but also results in the production and excretion of protein, as reflected by increased plasma concentrations. Taken together, these results highlight the usefulness of this model to measure transcriptional effects of renal IP.

**DISCUSSION**

Renal protection from ischemia by IP is currently an area of intense investigation. Particularly the use of genetically engineered animals may provide additional insight into molecular mechanisms of renal protection by IP. As technical details may significantly alter the experimental results, we performed a systematic evaluation of a novel model for isolated renal artery occlusion in mice, which specifically avoids the use of clamping or suturing the renal pedicle. In the present study, we demonstrate time-dose-dependent and highly reproducible renal injury with ischemia. Moreover, when this new...
model is compared with conventional clamping of the whole pedicle, renal protection by IP is more profound and more reliable. Finally, we also demonstrated that this model can be used for the investigation of gene regulation by IP, as erythropoietin message and protein levels were induced by IP.

Taken together, the present study provides feasibility of the hanging-weight system for isolated renal artery occlusion during IP, minimizing the variability and limitations associated with clamping or knot-based occlusion of the renal pedicle. In fact, this technique may be useful in further studies on renal protection by IP in mice.

Several studies have used renovascular pedicle clamping for renal protection with IP (3). In fact, one might argue that pedicle clamping is superior to isolated renal artery occlusion as potential surgical complications of dissecting away the renal artery from the renal vein are avoided and the surgical time involved with renal IP may be less. In contrast, the present study found renal-protective effects by IP only with isolated renal artery occlusion, while IP with renal pedicle clamping failed to increase renal resistance to ischemia. This is most likely related to the fact that clamping of the renal vein in addition to the renal artery results in venous outflow obstruction of the kidney. In fact, we observed a change in color from bright red to dark blue while applying the renal clamp to both vessels. In addition, the kidney became edematous and swollen. In fact, these changes did not disappear completely during short periods of reperfusion, as used during IP. This is also reflected by measurements of kidney function (e.g., creatinine clearance), which were further impaired by pedicle clamping, in contrast to isolated renal artery occlusion. Moreover, when

Fig. 7. Comparison of renal protection from ischemia by IP using isolated renal artery occlusion or pedicle clamping. Mice underwent right nephrectomy, and IP was performed using the hanging-weight system for isolated renal artery occlusion with 4 IP cycles (4-min ischemia, 4-min reperfusion) before 30 min of ischemia. Alternatively, the same IP protocol was used with simultaneous clamping of the renal artery and vein (pedicle clamping). Following the surgery, mice were placed in metabolic cages over 24 h. Plasma creatinine levels (A), plasma potassium (K⁺; B), creatinine clearance (C), urinary flow rate (D), urinary sodium (Na⁺; E) excretion, and urinary potassium (K⁺; F) excretion were determined. Results are means ± SD derived from 7–9 animals/condition. P values indicate differences between –IP and +IP mice. Note the absence of renal protection by IP following pedicle clamping.
the hanging-weight system is used, atraumatic dissection of the renal artery can be achieved quickly and with technical ease when the vessel is approached close to its take-off from the aorta where the renal artery and vein run independently. Taken together, we think that isolated renal artery occlusion for IP is superior to clamping of the pedicle, as it does not interfere with venous drainage of the kidneys.

Similar to the present study, previous investigations have demonstrated renal protection by IP in mice (3, 26, 29). Moreover, several studies have used genetically engineered mice in renal ischemia models. For example, several studies have demonstrated a critical role of adenosine signaling during renal ischemia and reperfusion (18–21). In fact, a series of very carefully executed studies performed renal ischemia and reperfusion in mice lacking T and B cells to determine tissue specifically whether adenosine 2A receptors are involved in renal protection from ischemia and reperfusion injury (8). These studies revealed that CD4+ T lymphocytes play an important role in adenosine 2A receptor-mediated tissue protection. Using adoptively transferred T cell- or B cell-deficient mouse models, further work revealed that IFN-γ appears to be a critical mediator for this complex interplay (9).

Table 1. Mean arterial pressure

<table>
<thead>
<tr>
<th>Anesthesia</th>
<th>IP</th>
<th>Ischemia</th>
<th>Reperfusion</th>
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<tr>
<td>−IP (n = 5)</td>
<td>103±8</td>
<td>102±8</td>
<td>112±7</td>
</tr>
<tr>
<td>+ IP (n = 5)</td>
<td>101±8</td>
<td>96±12</td>
<td>113±8</td>
</tr>
</tbody>
</table>

Values are means ± SD expressed in mmHg. n, No. of animals; IP, ischemic preconditioning. No significant differences between the −IP/+IP groups and between the different time periods were found.

Fig. 8. A: model displaying the experimental set-up for investigating gene regulatory effects of IP. B: renal erythropoietin (EPO) mRNA is induced by IP. Mice underwent right nephrectomy followed by IP treatment of the remaining kidney using the hanging-weight system for isolated renal artery occlusion [4 IP cycles (4-min ischemia, 4-min reperfusion) followed by indicated times of reperfusion (0–120 min)]. Sham-operated animals were used as control (CON). After indicated reperfusion time, total RNA was isolated followed by DNase digestion and conversion to cDNA. EPO mRNA levels were determined by real-time RT-PCR. Results are means ± SD calculated relative to β-actin and expressed as fold-change in transcript levels relative to control animals; n = 4 animals/group. P values indicate differences between sham-operated and preconditioned animals with different reperfusion times. C: plasma EPO levels were measured following right nephrectomy followed by IP treatment of the remaining kidney using the hanging-weight system for isolated renal artery occlusion (4 IP cycles consisting of 4 min of ischemia and 4 min of reperfusion) followed by 24-h of reperfusion time. Values are calculated as fold-change in EPO plasma levels with IP treatment; n = 6 animals/group. P indicates differences between −IP and +IP mice.
Despite many advantages associated with the use of gene-targeted mice to study renal protection by IP, some limitations of this approach have to be pointed out. While it is likely that the use of genetically modified mice may yield important information about IP, biological compensation for gene deletion is known to occur (11, 23). Moreover, it is well appreciated that different responses to renal ischemia have been observed in models with different genetic backgrounds (2, 4). For example, a recent study searched for an in vivo model of intrinsic resistance to renal ischemia, as this would provide an invaluable tool for investigating putative protectors from renal injury. This study showed that Brown-Norway rats are virtually resistant to renal ischemic injury, thus providing a novel model for studying mechanisms of renal protection from acute renal failure (2). Moreover, some evidence even suggests differences in responses between different ethnic groups (17). Such studies highlight that despite multiple similarities, molecular mechanisms and potential therapeutic targets identified in murine models cannot be directly transferred to a clinical scenario, but first require further testing in other models and settings.

An additional limitation of the present study is its focus on primary outcome parameters of renal injury and protection, such as plasma creatinine, creatinine clearance, urinary flow rate, or electrolyte status. To further unravel mechanisms of renal protection by IP, experimental approaches may need to be integrated that allow a more detailed examination of renal function and physiology during IP. For example, it is known that adenosine mediates renal vascular responses elicited by changes in NaCl concentration in the kidneys in the macula densa region of the nephron. In fact, mice deficient in the ecto-5'-nucleotidase (CD73, extracellular conversion of AMP to adenosine) showed impaired tubuloglomerular feedback regulation of the glomerular filtration rate, suggesting that extracellular adenosine generation at the glomerular afferent is largely CD73 dependent (5). Other studies revealed that adenosine generated by cd73-dependent and -independent mechanisms participates in the mediation of tubuloglomerular feedback in vivo (15). Thus studies on how renal IP modulates details of tubular and glomerular function, e.g., by using micropuncture techniques or renal hemodynamic measurements, may yield additional insight into renal protection by IP.

In summary, the present study describes a novel technique for performing renal IP in an intact murine model using a hanging-weight system for renal artery occlusion. In fact, this study demonstrates highly reproducible injury and renal protection by IP, thus providing more reliable renal protection by IP in mice and minimizing the variability associated with clamping of the renal pedicle.

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