Post-conditioning by a short administration of desflurane reduced renal reperfusion injury after differing of ischaemia times in rats

D. Obal1,4 *, K. Rascher2, C. Favoccia1, S. Dettwiler1 and W. Schlack3

1Department of Anaesthesiology and 2Department of Anatomy II, Heinrich-Heine University, Duesseldorf, Germany. 3Department of Anaesthesiology, Academical Medical Center, University of Amsterdam, The Netherlands. 4Department of Anesthesiology and the Outcomes Research Institute, University of Louisville, KY 530 S Jackson S, Louisville 40202, USA

*Corresponding author. E-mail: d.obal@louisville.edu

Background. ‘Anaesthetic post-conditioning’, that is administration of anaesthetics during early reperfusion, is known to have positive effects on several organs. For the kidney, however, the effects of post-conditioning by volatile anaesthetics are not well researched. We examined renal function and morphology after post-conditioning by desflurane.

Methods. Anaesthetized rats were subjected to 30 or 45 min of renal ischaemia 14 days after contralateral nephrectomy. Post-conditioning was achieved by administration of 1 MAC desflurane (6.7 vol%) for 15 min during early reperfusion (all groups \( n = 8 \)). Cystatin C (CyC), creatinine clearance (ClCr) and fractional sodium excretion (FENa) were measured in the awake rats over 3 days. Cell damage was graded from 1 to 4 in histological sections. Functional variables [mean (SD)] were compared statistically by a one-way ANOVA followed by Bonferroni’s multiple comparison test and histological scores (median and range) by Kruskal–Wallis test followed by Dunn’s multiple comparison test.

Results. Pre-ischaemia function did not differ between the groups, but was markedly reduced after ischaemia. After 30 min ischaemia, the area under the curve (AUC) for ClCr was smaller in the desflurane than in the control group \( [21.5 (5.0) \text{ vs } 31.6 (5.1) \text{ ml min}^{-1} \text{ h}] \ P < 0.05 \). After 45 min desflurane reduced the AUC compared with the control group for both CyC \( [15 (4) \text{ vs } 21 (3) \text{ mg litre}^{-1} \text{ h}] \) and FENa \( [1054 (22) \text{ vs } 1570 (572)\% \text{ h}] \) both \( P < 0.05 \). Morphological differences were greater between the 30 min groups [control: 2.75 (2.0–3.5) vs desflurane: 1.5 (1.0–2.5); \( P < 0.05 \)] than between the 45 min groups [control: 3.5 (3.0–4.0) vs desflurane: 3.0 (1.5–4.0)].

Conclusion. Desflurane post-conditioning protects renal function and tissue. This protection was greater after the short episode than after the long episode of ischaemia.

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Ischaemic injury of the kidney may lead to acute renal failure, which accounts for high mortality and morbidity in patients.1 An increasing number of studies suggest that volatile anaesthetics have a ‘pre- or post-conditioning’ effect when given shortly before or shortly after an ischaemic insult. These findings have been collected for the heart, not only in experimental animals but also in patients undergoing coronary artery bypass surgery.1,4

Although some of these reports postulate5,6 that volatile anaesthetics provide ‘peri-ischaemic’ protection of the kidney, to date no information is available on the effect of anaesthetic post-conditioning, that is, administration of a volatile anaesthetic at the beginning of reperfusion. Lee and colleagues5 demonstrated that volatile anaesthetics have a beneficial effect on renal function and preservation of structure when applied during ischaemia and the first 3 h of reperfusion. Their administration protocol does not allow...
differentiation between an anti-ischaemic and a specific effect reducing reperfusion injury (i.e. post-conditioning) and in addition the long administration time may not reflect the situation in clinical practice. The present study compares functional and morphological conditions after 30 or 45 min of renal ischaemia and examines whether or not a short period (15 min) of desflurane administered immediately after ischaemia protects against renal injury.

Material and methods

The study was performed in accordance with the regulations of German Law for the Protection of Animals and was approved by the Bioethics Committee of the District of Duesseldorf.

Animal preparation

Animal preparation was performed as described recently.22 35 anaesthetized Wistar rats [males; body weight 272 (6) g, mean (SD); S-ketamine, 100 mg kg\(^{-1}\), i.p.] underwent ischaemia/reperfusion experiments 14 days after right-sided nephrectomy. The single kidney model was used to avoid compensation of renal function by the normal kidney. Rats were anaesthetized with S-ketamine (100 mg kg\(^{-1}\)) and the lungs ventilated through a tracheal tube (small animal ventilator: Rhema-Labortechnik, Typ 10 ml, Class 34931, Germany) with a \(F_{\text{102}}\)~0.4 and a positive end expiratory pressure of 3–5 cm H\(_2\)O to maintain arterial pH, \(P_{\text{CO2}}\), and \(P_{\text{O2}}\) within the physiological range. Body temperature was maintained at 38.8°C by placing the rats on a heating pad.

A central venous catheter was implanted in the right external jugular vein for repeated blood sampling before ischaemia and during reperfusion. Through a dorsal approach the left renal pedicle was exposed and a ligature was placed around the artery and vein. The artery was occluded by tightening the snare 10 min after i.v. administration of heparin (150 u kg\(^{-1}\)). The disappearance of surface cyanosis was taken as evidence of successful reperfusion. The layers of the abdominal wall were closed with 4.0 polypropylene sutures and the skin infiltrated with local anaesthetic for postoperative analgesia [bubivacain 0.5% (0.5 mg)]. One day before ischaemia, the rats were placed in metabolic cages for assessment of renal function.

Experimental programme

Animals were randomly assigned to one of the following groups (n=8 in each group, Fig. 1) and maintained under general anaesthesia with S-ketamine (3 mg kg\(^{-1}\) min\(^{-1}\)):

**Control groups.** Fifteen minutes after the end of preparation (stabilization period), ischaemia was induced by tightening the snare around the renal vessels for 30 min (CON30) or for 45 min (CON45). After removing the snare and closing the abdominal cavity, the rats were recovered from anaesthesia. They were then returned to metabolic cages for 3 days.

**Desflurane ‘post-conditioning’ groups.** After the stabilization period, the vessels were occluded for 30 min (DES30) or for 45 min (DES45). Three minutes before renal reperfusion, S-ketamine administration was discontinued and desflurane (Baxter Deutschland, GmbH, Unterschleissheim, Germany) administration initiated (6.7 vol% corresponding to one MAC\(^{6}\)). The concentration was increased stepwise in order to avoid haemodynamic side-effects.\(^{7,8}\) After 15 min, desflurane administration was terminated and the abdominal cavity closed. Following complete recovery from anaesthesia, rats were returned to metabolic cages for 3 days.

Renal function

All animals were housed in a controlled environment room [22 (2)°C, illuminated from 07.00 to 18.00 h] and had free access to food pellets and water. Fluid intake and urine output were measured. Blood samples (1.3 ml) were obtained at 1, 24, 48 and 72 h after the beginning of reperfusion. Creatinine (mg ml\(^{-1}\)) clearance (\(\text{Cl}_{\text{Cr}}, \text{ml min}^{-1}\)) was calculated by the standard formula and used similar to cystatin C as variable of glomerular function. Fractional sodium excretion (\(\text{FE}_{\text{Na}}, \%\)) was calculated as (urine\(_{\text{Na}}\) · serum\(_{\text{Na}}\))/(serum\(_{\text{Cr}}\) × urine\(_{\text{Cr}}\))×100 as a variable of tubular function. All electrolyte concentrations were measured in a flame photometer (Eppendorf, EFOX5053, Germany). Creatinine values were measured by a modified Jaffé-reaction, based on a photometrically measured colour reaction (Cobas Mira S).

Histology

After 3 days of reperfusion the animals were deeply anaesthetized and the kidneys excised by a mid-abdominal approach. Thick slices of kidney tissue were fixed in Bouin’s solution, dehydrated in a graded series of alcohol and embedded in paraffin. Four micrometre sections were processed with periodic acid-Schiff (PAS) reagent and haematoxylin. Three sections lying at least 30 sections
apart were assessed by two observers blinded to treatment. Histological cell damage was graded as previously described.\textsuperscript{9} Criteria of cell damage were loss of brush border, flattening of tubular epithelial cells, dilation of tubules and tubular congestion with protein casts. These criteria were slightly modified from those introduced by Jablonski and colleagues.\textsuperscript{10}

**Immunohistochemical detection of ED1-positive cells**

ED1 macrophage infiltration was detected immunohistochemically. Four micrometre thick paraffin sections were dewaxed in xylene and rehydrated in a graded series of alcohol. The sections were incubated with proteolytic enzyme for 5 min (Dako, Hamburg, Germany), rinsed in TBS (0.05 M Tris buffered saline, pH 7.4) and then covered with 3% hydrogen peroxide for 20 min to block endogenous peroxidase. After washing in TBS, the slides were coated with the primary antibody 1:500 (ED1 mouse anti rat CD68, MCA341R, Serotec, Oxford, UK) for 16 h at 4°C\textsuperscript{14}. The slides were rinsed with TBS and then incubated with MACH 3 mouse probe for 15 min and rinsed with TBS followed by incubation in MACH 3 HRP for 15 min at room temperature (both reagents from Biocare Medical, Walnut Creek, CA, USA). The product reaction was seen by incubation in diaminobenzidine reagent (Dako, Hamburg, Germany) for 10 min at room temperature and nuclei subsequently stained with hematoxylin.

**Statistical analysis**

All nominally distributed data are expressed as mean values (SD), ordinally distributed values as results of the histological examination are expressed as median and range.

For statistical analysis of FE\textsubscript{Na}, cystatin C and Cl\textsubscript{Cr} the areas under the curves were calculated and differences between desflurane and control groups analysed by a one-way ANOVA followed by Bonferroni’s multiple comparison test. The histological scores for cell damage were compared by the Kruskal–Wallis test followed by Dunn’s multiple comparison test (PRISM 4.0 GraphPad Software, Inc., San Diego, USA). \(P\)-values of less than 0.05 were considered significant.

**Results**

Experiments were performed in a total of 35 animals. Three animals were excluded from histological and functional analysis. In the CON45 group two rats had neither a significant increase in plasma creatinine nor morphological cell damage, indicating incomplete artery occlusion. One rat of the DES45 group had incomplete reperfusion with corresponding macroscopic findings paralleled by an unrealistic increase (an aberration of more than twice standard deviations in creatinine values and sodium excretion ratio combined with a nearly complete loss of urine production). Complete datasets were collected for all the other rats.

**Renal function**

After right-sided nephrectomy, renal function remained within the normal baseline range in all groups before ischaemia/reperfusion experiments. Both 30 and 45 min of renal artery occlusion was followed by a decline in renal function as assessed by blood and urine analysis (Table 1; Figs 2–4). However, renal function was poorer after 45 min than after 30 min of ischaemia. Twenty-four

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Pre-ischaemia</th>
<th>Reperfusion</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>Creatinine (mg dl\textsuperscript{-1})</td>
<td>CON30</td>
<td>0.6 (0.1)</td>
<td>1.0 (0.1)</td>
</tr>
<tr>
<td></td>
<td>DES30</td>
<td>0.7 (0.1)</td>
<td>0.9 (0.2)</td>
</tr>
<tr>
<td></td>
<td>CON45</td>
<td>0.5 (0.1)</td>
<td>0.8 (0.2)</td>
</tr>
<tr>
<td></td>
<td>DES45</td>
<td>0.8 (0.1)</td>
<td>0.9 (0.1)</td>
</tr>
<tr>
<td>Cl\textsubscript{Cr} (ml min\textsuperscript{-1})</td>
<td>CON30</td>
<td>1.03 (0.12)</td>
<td>0.07 (0.03)</td>
</tr>
<tr>
<td></td>
<td>DES30</td>
<td>0.87 (0.17)</td>
<td>0.16 (0.06)</td>
</tr>
<tr>
<td></td>
<td>CON45</td>
<td>1.16 (0.18)</td>
<td>0.02 (0.01)</td>
</tr>
<tr>
<td></td>
<td>DES45</td>
<td>0.96 (0.33)</td>
<td>0.03 (0.01)</td>
</tr>
<tr>
<td>FENa (%)</td>
<td>CON30</td>
<td>0.9 (0.2)</td>
<td>13.1 (7.5)**</td>
</tr>
<tr>
<td></td>
<td>DES30</td>
<td>0.7 (0.2)</td>
<td>6.6 (3.7)</td>
</tr>
<tr>
<td></td>
<td>CON45</td>
<td>0.7 (0.2)</td>
<td>48.8 (12.4)**</td>
</tr>
<tr>
<td></td>
<td>DES45</td>
<td>1.1 (0.4)</td>
<td>26.8 (17.0)</td>
</tr>
<tr>
<td>Urine Osmolarity (mmol litre\textsuperscript{-1})</td>
<td>CON30</td>
<td>1150 (373)</td>
<td>484 (117)</td>
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<tr>
<td></td>
<td>DES30</td>
<td>1309 (358)</td>
<td>593 (191)</td>
</tr>
<tr>
<td></td>
<td>CON45</td>
<td>1416 (242)</td>
<td>493 (192)</td>
</tr>
<tr>
<td></td>
<td>DES45</td>
<td>1270 (361)</td>
<td>411 (70)</td>
</tr>
<tr>
<td>Fluid balance (ml 24 h\textsuperscript{-1} 100 g\textsuperscript{-1})</td>
<td>CON30</td>
<td>10 (3)</td>
<td>0 (5)</td>
</tr>
<tr>
<td></td>
<td>DES30</td>
<td>9 (5)</td>
<td>-1 (5)</td>
</tr>
<tr>
<td></td>
<td>CON45</td>
<td>8 (3)</td>
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</tr>
<tr>
<td></td>
<td>DES45</td>
<td>8 (2)</td>
<td>2 (5)</td>
</tr>
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hours after renal artery occlusion \( \text{FENa} \) increased more in CON45 (49 (12)%%) than in CON30 (13 (8)%%). At the same timepoint, glomerular filtration rate was markedly reduced in the 45 min group as compared with the 30 min ischaemia group, indicated by a reduction in ClCr [CON45: 2.8 (0.7) ml min\(^{-1}\) vs CON30: 2.4 (0.3) ml min\(^{-1}\)]. Desflurane had a positive effect on both glomerular and tubular function. There was less impairment of renal function in the treated animals. Areas under the curves were smaller in the desflurane group than in the control group for ClCr [21.5 (5.0) vs 31.6 (5.1) ml min\(^{-1}\) h, \( P<0.05 \)] after 30 min and for \( \text{FENa} \) [1054 (221) vs 1570 (572)% h] and cystatin C [15 (4) vs 21 (3) mg litre\(^{-1}\) h] after 45 min ischaemia. After ischaemia, creatinine concentrations increased in all groups (Table 1), with the highest levels found in the untreated 45 min group [4.1 (0.5) mg ml\(^{-1}\)]. This increase in creatinine was less pronounced in both treatment groups after 48 h of reperfusion [DES45: 2.7 (1.2) mg ml\(^{-1}\), \( P<0.01 \)]. The kidneys lost the ability to concentrate urine after ischaemia and reperfusion. The osmolarity of the urine decreased and all animals had a disturbed fluid balance after 24 and 48 h of reperfusion (Table 1). The rats subjected to 30 min of ischaemia regained almost the pre-ischaemic capacities to concentrate urine by the end of the experiment. Recovery in the
45 min groups was less pronounced, but desflurane treatment had a positive effect on the ability to concentrate urine [CON45: 0 (2) ml 24 h⁻¹ 100 g⁻¹ vs DES45: 7 (5) ml 24 h⁻¹ 100 g⁻¹, P<0.05].

Histological examination
Both 30 and 45 min of renal ischaemia were followed by the destruction of renal tissue, especially at the outer medullary stripe and the cortico-medullary border zone. Figure 5A shows the outer medullary stripe in a sham-operated animal. Epithelial cells of the S3 segment have intact brush borders and the tubules are not distended. Cell injury at level 1 is illustrated in Figure 5C: many cells have lost their brush borders and some tubules are distended, others slightly congested. Many tubules at level 4 injury (Fig. 5E) contain casts of densely packed cell debris. The epithelial cells are flattened and some tubules completely denuded of cells, exposing the basement membrane. Semi-quantitative assessment demonstrated that the degree of renal tubular cell damage was more severe in the groups of animals exposed to 45 min of ischaemia than in those exposed to 30 min of ischaemia (Fig. 6). However, some animals displayed heterogeneous patterns of injury and classifying these animals to a specific level of injury was done on the basis of the level predominating in the sections. Desflurane reduced the level of cell damage in both treatment groups, the degree of reduction was greater after 30 min [DES30: 1.5 (1.0–2.5) vs CON30: 2.75 (2.0–3.5), P<0.05] than after 45 min of ischaemia [DES45: 3.0 (1.5–4.0) vs CON45: 3.5 (3.0–4.0), Figure 6].

Immunohistochemical detection of ED1-positive cells
Renal ischaemia was followed by an infiltration of ED1-positive cells. The number and the distribution pattern of positive cells were different between 30 and 45 min of ischaemia. Variations in distribution pattern, generally corresponding to the variations in levels of tubule injury seen in the PAS sections described above, were also detected. Figure 5B shows isolated positive cells in a sham-operated animal. Animals with lower levels of tubular injury had positive cells scattered across the entire cortico-medullary border. They were located in the peritubular spaces and only rarely within the tubules (Fig. 5D). This pattern was most common in the groups of animals subjected to the shorter period of ischaemia. In animals with higher levels of injury, positive cells were often densely clustered around tubules and often found in the tubule lumen (Fig. 5F). This pattern of distribution was seen most often in the animals subjected to longer periods of ischaemia.

Discussion
The major finding of our study was that a 15 min application of desflurane at the beginning of reperfusion reduces ischaemia/reperfusion injury in the kidney. This effect was more pronounced after 30 min than after 45 min of renal artery occlusion.

It is well known that administration of volatile anaesthetics reduces myocardial reperfusion injury, even after administration of cardioplegic solutions. In contrast, there is much less information available on the potential protective effects of anaesthetic post-conditioning in the kidney, for example protection by the administration of an anaesthetic during early reperfusion. Our experiments were designed to examine the effects of a short post-ischaemic application of desflurane on the functional and morphological integrity of the rat kidney. To determine whether the effects of post-conditioning by desflurane are dependent upon the duration of ischaemia, periods of 30 or 45 min of artery occlusion were chosen.
In this model the single kidney may be more susceptible to injury than when an unaffected kidney compensates, especially as male rats are known to be more susceptible than females to ischaemia/reperfusion injury. This model produces a severe, but not fatal injury and has been used in numerous earlier studies. The most severely affected part of the kidney is the outer medullary stripe. The degree of ischaemia/reperfusion injury correlated with the duration of the ischaemic insult on a group level. However, the extent of cell damage seen in both the PAS sections and in ED1 immunostaining was heterogeneous in some animals. Sections which had a distinct area of high level injury next to one with a lower level of injury suggest that reperfusion did not always take place evenly. The degree of cell injury also

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**Fig 5** Details of the outer medulla. (A, C, E) Periodic acid-Schiff (PAS) reaction; bar 50 μm; (B, D, F) ED1 immunostaining, cortex towards upper edge of micrographs; bar 50 μm. Sham operated: (A) Brush borders (B) and cells of proximal tubules are intact; distal tubules (D). (B) ED1-positive cells (arrows) are rarely found only in the interstitial spaces or in vessels. Level 1 injury: (C) Most brush borders (B) are detached and float in the tubule lumen; distal tubules (D). (D) ED1-positive cells (arrows) are scattered in interstitial spaces but rarely within tubules. Level 4 injury: (E) Dense casts of cell debris clog distended proximal tubules; some casts also contain nuclear material, nuclei, or both (B). (F) Densely clustered ED1-positive cells within and surrounding some tubules (asterisk); others have no marked cells.
likely to be the most severely damaged areas of the outer medulla would be expected. Cells which were less severely damaged would most likely be removed by apoptosis and would not attract large numbers of macrophages. We administered desflurane for only 15 min, a much shorter period than in the study by Lee and colleagues, who applied volatile anaesthetics during ischaemia and over a total time of 3 h of reperfusion. Even this long period of desflurane administration did not alter the expression of pro-inflammatory messenger RNA in renal cortical slices. Therefore, it seems unlikely that desflurane produced immune-modulatory effects in our animals. The short time of desflurane administration makes it more likely that mechanisms at the very beginning of reperfusion are responsible for the protective effect.

Toxic oxygen metabolites have been postulated to contribute to renal ischaemia/reperfusion injury, but their biochemical assessment and contribution as related to the duration of ischaemia is unclear. Dobashi and colleagues demonstrated that the loss of antioxidant enzymes increased with the duration of ischaemia. The degree of ischaemic injury seemed to correlate with the amount of unbound oxygen radicals. They further suggested that the heterogeneous pattern of renal damage might be in agreement with the differential distribution of antioxidant enzymes in different cell types in the kidney. Whether or not desflurane leads to an antioxidant effect during early reperfusion in the kidney deserves to be examined, but Glantz and colleagues achieved myocardial protection by completely inhibiting hydroxyl production by administering halothane during early reperfusion.

In our chronic model we did not measure sympathetic nerve activity nor renal blood flow. It is generally known that a rapid increase in the concentration of desflurane might influence the autonomous nervous system and might increase sympathetic activity, which might result in increases in mean arterial blood pressure and perhaps a slight decrease in renal blood flow. We avoided sympathetic activation by stepwise increases in inspired desflurane concentration but cannot exclude that alterations of renal blood flow at the onset of reperfusion may have been beneficial in reducing injury in our desflurane-treated animals. Haab and colleagues demonstrated that the...
kidney tolerates controlled reperfusion much better than rapid reperfusion and this finding is very similar to observations made in the heart, where staged reperfusion also reduced myocardial cell damage after ischaemia.39

We used FENa as a variable of tubular function. An earlier report suggests that ischaemia/reperfusion injury leads to a pronounced downregulation of sodium transporters and the sub-cellular relocation of Na/K/ATPase pumps.40 Natriuresis occurs as a result. The differences in creatinine clearance and the more sensitive variables FENa and cystatin C41 probably reflect differences in the degree of cell damage after 30 and 45 min of ischaemia. Kidneys with moderate cell damage as seen after the shorter period of ischaemia showed improvements in renal function and differences in creatinine clearance between the desflurane and control groups. Severe cell damage as seen in the 45 min groups caused a deterioration of glomerular filtration rate and only the highly sensitive variables such as FENa and cystatin C were able to detect significant differences in those groups.

The present study has demonstrated for the first time that a short period of 1 MAC desflurane administration during early reperfusion (post-conditioning) protects against ischaemia/reperfusion injury, with the extent of protection depending on the duration of ischaemia.

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References

23 Feng J, Lucchetti E, Ahuja P, Pasch T, Perriard JC, Zaugg M. Isoflurane postconditioning prevents opening of the mitochondrial permeability transition pore through inhibition of glycogen synthase kinase 3β. Anesthesiology 2005; 103: 987–95
27 Yamashita J, Itoh M, Kuro T, et al. Pre- or post-ischemic treatment with a novel Na+/H+ exchange inhibitor, KB-R7943, shows renal
protective effects in rats with ischemic acute renal failure. J Pharmacol Exp Ther 2001; 296: 412–19
28 Haworth RA, Goknur AB. Inhibition of sodium/calcium exchange and calcium channels of heart cells by volatile anesthetics. Anesthesiology 1995; 82: 1255–65
32 Jose MD, Ikezumi Y, van RN, Atkins RC, Chadban SJ. Macrophages act as effectors of tissue damage in acute renal allograft rejection. Transplantation 2003; 76: 1015–22
35 Muse KE, Oberley TD, Sempf JM, Oberley LW. Immunolocalization of antioxidant enzymes in adult hamster kidney. Histochem J 1994; 26: 734–53