Effects of different perfusates on functional parameters of isolated perfused dog kidneys

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Abstract

Background. The isolated perfused canine kidney has been established as a valid model for conducting both renal physiology and transplantation research. This model is of particular importance for developing new strategies to improve graft function after renal transplantation. In the present study, a newly developed method using isolated haemoperfused porcine kidneys was adapted for use in canine kidneys. In contrast to haemoperfusion, synthetic perfusion media can be standardized and can prevent the initiation of blood-mediated reperfusion reactions. Thus, an additional aim was to determine whether blood could be replaced by synthetic cell-free perfusion solutions.

Methods. Canine kidneys (n = 30) were harvested from donors euthanized in veterinary practices for causes unrelated to the present study. The kidneys were isolated and perfused with autologous blood or cell-free synthetic electrolyte buffer (Tyrode solution). During perfusion, we monitored renal perfusate flow (RPF), glomerular filtration rate (GFR), electrolyte and glucose reabsorption, oxygen consumption and urine concentration.

Results. Changes in perfusion medium did not affect the RPF. In contrast, GFR, urine concentration and oxygen consumption were significantly higher, whereas fractional excretion of sodium and glucose were significantly lower in blood- than in Tyrode-perfused kidneys.

Conclusions. This system offers a simple model for studying whole-organ functional alterations after acute renal ischaemia. Renal function indicators were below values reported during in vivo physiological conditions. These functions were better conserved when kidneys were perfused with autologous blood than with Tyrode.

Keywords: dog; functional state; haemoperfusion; isolated perfused kidney; perfusion solutions

Introduction

As the most frequent whole-organ transplantation worldwide, kidney transplants have been established in recent years as routine treatment for various severe renal diseases [1]. However, dysfunction of transplanted organs, which probably derives from numerous factors [2], is a common complication that limits prognosis. The development of appropriate techniques to minimize organ ischaemia and reperfusion injuries may lead to improvements in graft organ function and prognosis [3].

The isolated perfused kidney (IPK) has been established as a valid model for investigating renal functions [4–8], and may also be of great value for assessing preservation techniques [9]. Recently, a method has been developed that uses porcine slaughterhouse kidneys for IPK studies [10]. This method is based on harvesting organs during the commercial slaughter process, subsequent cold preservation and normothermic reperfusion with autologous blood. This technique is of value for transplantation research because it uses controlled renal perfusion conditions while providing an alternative to animal experiments.

In the present study, the newly developed isolated haemoperfused porcine kidney method was adapted for use in canine kidneys. Since experiments in living animals, particularly in dogs, are under increasing public criticism, these studies are of value because they allow a species-specific link between existing renal function data from canine studies and newly established IPK experiments. In particular, we studied the effects of different perfusates on renal function in order to determine whether blood as the perfusion medium...
could be replaced by simple synthetic solutions in IPK experiments. These solutions are of interest because they are standardizable and can prevent the initiation of blood-mediated reperfusion reactions [11].

Subjects and methods

Animals

Dogs (n = 17, different breeds, 7.6 ± 4.9 years of age, 16.2 ± 9.5 kg body mass) that had been euthanized in veterinary practice for miscellaneous reasons unrelated to our study served as kidney donors. We obtained owners' consent and permission from the local veterinary authorities. The animals did not show clinical signs of renal disease.

Kidney harvesting and preservation

After anaesthesia with 25–35 mg/kg pentobarbital (Narcoren®; Merial GmbH, Germany), the kidneys were removed and immediately stored on ice. Median native kidney mass was 39.1 g (27.9–61.4 g). The median duration of warm ischaemia was 10 min (7–15 min). The kidneys were arterially flushed with 20 ml of cold (warm ischaemia was 10 min (7–15 min). The kidneys were removed and immediately stored on ice. Median native kidney mass was 39.1 g (27.9–61.4 g). The median duration of warm ischaemia was 10 min (7–15 min). The kidneys were arterially flushed with 20 ml of cold (warm ischaemia was 10 min (7–15 min). The kidneys were immediately connected to the reperfusion system and was immed iately connected to the reperfusion system for 6.5–8.0 h before use of the second kidney.

Reperfusion system

The reperfusion system has been described in detail elsewhere [10]. In brief, either autologous blood or Tyrode solution (both with additives described in the following section) was recirculated continuously as perfusate through the kidney with a constant pressure of 13.4 ± 0.7 kPa. The perfusate was dialyzed against a 10-fold volume of Tyrode solution (= dialysate). Both circuits were interconnected via a commercial low flux module for dialysis (Hemoflow F7 HPS, Fresnius Medical Care, Bad Homburg, Germany), enabling gas exchange between perfusate and dialysate, and dialysis of the perfusate. The dialysate was oxygenated, and carboxygenated for pH control.

Mean arterial blood pressure (MAP) was measured continuously and was controlled through adjusting the pump speed (MC 360, Ismatec SA, Glattbrugg–Zürich, Switzerland), from which renal perfusate flow (RPF) was calculated. Loss of perfusate by diuresis was compensated through a fluid shift from the dialysate to the perfusate circuit. Perfusion volume was monitored by weighing the perfusate reservoir continuously.

Laboratory analysis

Perfusate and urine samples were analysed for creatinine, Na and glucose using routine procedures at the Department of Clinical Chemistry.

Table 1. Perfusion groups and perfusion media used in the experiments

<table>
<thead>
<tr>
<th>Perfusion group</th>
<th>No. of kidneys</th>
<th>Preservation</th>
<th>Main perfusate</th>
<th>Additives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyr 1</td>
<td>8</td>
<td>Yes</td>
<td>Tyrode</td>
<td>No</td>
</tr>
<tr>
<td>Tyr 2</td>
<td>4</td>
<td>No</td>
<td>Tyrode</td>
<td>No</td>
</tr>
<tr>
<td>Tyr 3</td>
<td>5</td>
<td>Yes</td>
<td>Tyrode</td>
<td>4% BSA b, substrates c</td>
</tr>
<tr>
<td>bl 1</td>
<td>6</td>
<td>Yes</td>
<td>blood</td>
<td>No</td>
</tr>
<tr>
<td>bl 2</td>
<td>3</td>
<td>Yes</td>
<td>blood</td>
<td>50 g/l mannitol</td>
</tr>
<tr>
<td>bl 3</td>
<td>4</td>
<td>Yes</td>
<td>blood</td>
<td>Substrates c</td>
</tr>
</tbody>
</table>

aKidneys not preserved were connected to the reperfusion system immediately after organ harvesting. Preserved kidneys were treated with preservation solution and stored on ice for a certain period of time.

bBSA, bovine serum albumin fraction V (Biomol GmbH, Hamburg, Germany).

cSubstrates: amino acids 2.1 g/l (Aminosteril® 15%, Fresenius-Kabi, Austria, 28 ml/l), mannitol 5 g/l (Sigma-Aldrich), pyruvate 2 mmol/l (Roche Diagnostics), glutathione 1 mmol/l (Roche Diagnostics), α-tocopherol 151 U (Vitaselen, Selectavet, Germany), ascorbic acid 10 mg/l (Sigma-Aldrich).
Laboratory Medicine, Charité Campus Virchow. Perfusate gas analysis (PO₂, PCO₂, pH, as well as SO₂ and haemoglobin, for blood as the perfusate) was carried out with an ABL505 and OSM3 (Radiometer, Copenhagen, Denmark). Oncotic pressure was calculated from total protein concentration in the perfusate [12].

Data presentation and statistics
When appropriate, data are shown as means ± SD. Non-normally distributed data are presented as ‘medians (1st quartile–3rd quartile).’ For these data, box and whisker plots were used for graphical presentation. They indicate the median (solid line), first to third quartiles (box), values within a range of 1.5 times the interquartile range (whiskers), and extreme values. Statistical analyses were carried out using appropriate non-parametric tests (Kruskal–Wallis, Mann–Whitney, Wilcoxon and Friedman). Significance was defined as $P < 0.05$.

Results

Haemodynamics
During the urine collection periods, RPF and MAP were within constant ranges. RPF did not differ significantly between the perfusate groups, and the mean value for all groups was $0.96 \pm 0.56 \text{ ml/min/g}$ (Figure 2A). The oncotic pressure was $0.66 (0.33–0.91)$ kPa in the three blood groups (bl 1–3), 0 kPa in the two plain Tyrode groups (Tyr 1 and 2) and 1.21 (0.98–1.61) kPa in the Tyrode + bovine serum albumin (BSA) group (Tyr 3).

Glomerular filtration
There was a low correlation between RPF and GFR ($r = 0.3$, $P = 0.1$). In contrast to RPF, there were considerable GFR differences between perfusate groups as well as large variations within each group. The lowest GFR values were in both Tyrode groups (with and without BSA) that tested preserved kidneys.
Median GFR was >10 times higher in non-preserved than in preserved Tyrode-perfused kidneys (median creatinine clearance 22.8 μl/min/g in the Tyr 2 group vs 1.7 μl/min/g in the Tyr 1 group). Preserved blood-perfused kidneys (with and without BSA) showed GFR values that were similar to values in the Tyr 2 group. GFR values were therefore significantly higher (P ≤ 0.005) in the blood groups than in the preserved Tyrode groups (Table 2). Addition of BSA did not affect the GFR values.

Reabsorption of glucose
The absolute amount of reabsorbed glucose paralleled the values for GFR (r = 0.93, P = 0.01; Table 2). In order to evaluate tubular function relative to GFR, the fractional glucose excretion (FEglucose) was calculated (Figure 3). These data revealed a clear and statistically significant (P < 0.001) difference between Tyrode- and blood-perfused kidneys. Median FEglucose was 0.62 (0.45–1.16) in Tyrode-perfused compared with 0.30 (0.22–0.39) in blood-perfused kidneys. In contrast to differences in GFR and in filtered glucose load between preserved and non-preserved Tyrode-perfused kidneys, the FEglucose was only marginally lower in the non-preserved group. The addition of BSA, mannitol or substrates did not significantly influence relative glucose reabsorption.

Reabsorption of sodium
Sodium excretion was generally high and paralleled the results described for glucose, i.e. the absolute amount of reabsorbed sodium increased with filtered load (r = 0.96, P < 0.001; Table 2). Filtered sodium load was 2.61 (0.56–8.26) μmol/min/g in blood-perfused kidneys and 0.34 (0.11–1.54) μmol/min/g in Tyrode-perfused kidneys. Absolute sodium reabsorption was ~10 times higher in the blood-perfused groups [1.99 (0.30–5.28) μmol/min/g] than in the Tyrode-perfused kidneys [0.12 (0.03–0.55) μmol/min/g]. In agreement with GFR values, and therefore with filtered load, sodium reabsorption was significantly (P < 0.001) higher in blood-perfused kidneys [FEsodium = 0.29 (0.22–0.39)] than in the Tyrode groups [FEsodium = 0.62 (0.45–1.16)]. Finally, there was a tendency for lower sodium reabsorption in perfusate groups containing BSA and substrates compared with the respective groups not containing these substances (Figure 3).

Urine concentration
In the Tyrode groups, 83 ± 17% of the primary ultrafiltrate was excreted as terminal urine, contrasting with only 57 ± 17% in the blood-perfused groups (Figure 4). Within the blood group, differences between the subgroups were also found, with the highest urine concentration in kidneys perfused with plain, diluted blood.

Renal metabolism
Oxygen consumption was greater in the blood- than in the Tyrode-perfused groups (P < 0.001), which had median values of 0.79 (0.59–1.05) and 0.18 (0.09–0.50) μmol/min/g, respectively (Figure 5A). Whereas in the blood groups, the oxygen consumption/supply ratio

Table 2. Selected renal filtration, electrolyte and water transport, and metabolic parameters summarized for the three Tyrode- and three blood-perfused groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tyrode (Tyr 1–3)</th>
<th>Blood (bl 1–3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine clearance (μl/min/g)</td>
<td>1.76 (0.74–10.70)</td>
<td>14.66 (3.14–50.67)</td>
</tr>
<tr>
<td>Diuresis (μl/min/g)</td>
<td>1.45 (0.64–7.94)</td>
<td>5.62 (2.00–24.69)</td>
</tr>
<tr>
<td>Reabsorbed sodium (μmol/min/g)</td>
<td>0.12 (0.03–0.55)</td>
<td>1.99 (0.30–5.28)</td>
</tr>
<tr>
<td>Reabsorbed glucose (μg/min/g)</td>
<td>0.42 (0.07–2.17)</td>
<td>10.12 (1.43–57.62)</td>
</tr>
<tr>
<td>Arterial O2 supply (μmol/min/g)</td>
<td>0.30 (0.18–0.83)</td>
<td>3.21 (2.30–5.325)</td>
</tr>
<tr>
<td>Venous O2 return (μmol/min/g)</td>
<td>0.12 (0.07–0.23)</td>
<td>2.03 (1.37–4.56)</td>
</tr>
</tbody>
</table>

Data presented as medians (1st–3rd quartile).
was 26.7 ± 17.0%, it was 57.5 ± 15.9% in the Tyrode groups (Table 2). In order to evaluate whether oxygen supply to the kidney was the limiting factor for oxygen consumption, we recorded values of partial oxygen pressure in the arterial (PaO₂) and venous (PvO₂) perfusates (Figure 5B). We found that PaO₂ did not differ between blood- and Tyrode-perfused kidneys (P = 0.81). The overall PaO₂ was 40.9 ± 14.4 kPa. The median PvO₂ was 17.9 (10.2–20.0) kPa in the Tyrode groups and was 7.5 (4.3–13.0) kPa in the blood groups. The minimal PvO₂ attained was 3.1 kPa.

**Discussion**

The standards for kidney transplantation have improved significantly in recent years and have been paralleled by large numbers of renal transplantation studies using experimental animals [13–15]. The isolated canine kidney provides a useful approach compared with the more frequently used small animal models that have substantial disadvantages, which include organ size and function.

The present study examined the effects of different perfusates in a newly developed method of isolated haemoperfused canine kidneys, which was adapted from a porcine kidney perfusion model. Although six different subgroups of perfusates were used, the primary aim of the study was to compare Tyrode- with blood-perfused kidneys. In general, the blood-perfused groups performed significantly better than Tyrode-perfused groups, indicating that autologous blood perfusion is the preferred method. Although differences between the three Tyrode and blood subgroups (Tyr 1–3 and bl 1–3, respectively) were not significant, non-preserved Tyrode-perfused kidneys (Tyr 2) tended to perform (GFR, FEglucose) better than the Tyr 1 or Tyr 3 groups. The addition of additional substrates that may positively affect metabolism, such as pyruvate, amino acids or antioxidants (vitamins E and C, glutathione), to neutralize reactive oxygen species did not significantly improve renal functions.

RPF is the main determinant of GFR and therefore influences all successive renal function parameters [16]. In contrast to the theoretical expectation of a decreasing RPF with increasing perfusate viscosity [17], RPF was similar in all six perfusion subgroups. The low correlation between RPF and GFR is in accordance with previous IPK studies [18]. The significantly greater GFRs in the blood- than in the Tyrode-perfused kidneys were probably caused by changes in the ultrafiltration coefficient (Kf). This assumption is based on previously published studies showing that Kf decreased in response to low protein concentrations [19]. The mechanisms for these alterations in Kf are not yet known and it is unclear why the Tyr 3 group (Tyrode + 4% BSA) had GFRs that were similar to the Tyr 1 group (Tyrode without BSA).

The main driving force for electrolyte and glucose reabsorption is the sodium/potassium pump [17].
In general, there is a stoichiometry of 26–30 mmol of transported sodium per mmol of consumed O₂. This number refers to the direct and indirect exchange of sodium/potassium-dependent sodium transport [17]. The quantity of oxygen consumption in our experiments should have allowed reabsorption of all filtered sodium in both Tyrode- and blood-perfused kidneys. However, \( F_{\text{Na}} \) was significantly greater in Tyrode- than in blood-perfused kidneys. This discrepancy suggests that oxygen consumption was not the only limiting factor for sodium reabsorption in the present IPK model. This explanation is supported by the low coefficient of correlation between oxygen consumption and sodium reabsorption \( (r = 0.55, P = 0.002) \). Nonetheless, it is apparent that the perfusion groups with higher oxygen consumptions generally performed better.

Given that in vivo \( 60–70\% \) of the filtered sodium is reabsorbed in the proximal tubule [17], the present data suggest that sodium reabsorption in this part of the nephron was reasonably functional, at least for blood-perfused kidneys. The high coefficient of correlation between sodium and glucose reabsorption supports this conclusion, because glucose reabsorption is restricted exclusively to the proximal tubule and results from secondary active sodium/glucose co-transport [17].

The greater oxygen consumption in the blood-perfused groups may be due to the higher oxygen supply associated with haemoglobin-bound oxygen transport. It is well known that cellular oxygen transport using haemoglobin is in equilibrium with dissolved oxygen, allowing oxygen partial pressure \( (P_{O_2}) \) to be an indicator of oxygen supply \( (P_{O_2}) \) [20]. However, \( P_{O_2} \) levels were similar in Tyrode- and blood-perfused kidneys and were \( 4 \) times greater than normal in both groups. The Tyrode-perfused kidneys extracted \( 60\% \) (53–65\%) of delivered oxygen compared with 20\% (13–37\%) in the blood-perfused kidneys. The greater \( P_{\text{V}O_2} \) in the Tyrode- than in blood-perfused kidneys indicates that even with Tyrode perfusion there was an oxygen reserve after organ passage. In general, a \( P_{\text{V}O_2} \) of \( <4 \) kPa is considered an indicator of hypoxia [20], and this level was not attained in the Tyrode-perfused groups.

The total amount of reabsorbed water was significantly higher in the blood- than in Tyrode-perfused kidneys. Because water reabsorption is mainly coupled to solute reabsorption, this finding reflects a superior reabsorption of solutes in the blood-perfused kidneys. Under physiological conditions, the final urine concentration takes place in the collecting duct and requires antidiuretic hormone \( (\text{ADH}) \) [17]. Since ADH was not added to our perfusates, we did not expect that urine osmolality would be much greater than perfusate osmolality.

In conclusion, the isolated perfused canine kidney model used in the present experiments represents a useful approach for simulating isolated renal functions. This model may provide a useful means for studying renal ischaemia/reperfusion injuries as well as the effects of preservation solutions and storage conditions currently used for kidney transplantation. The present system offers a simple way to study whole-organ functional alterations after acute renal ischaemia. The functional capacities are therefore below the values found under physiological conditions in vivo. Preservation with autologous blood resulted in a better conservation of renal functions compared with perfusion with cell-free electrolyte buffer (Tyrode solution).

Acknowledgements. The authors appreciate the help of all members of the Isolated Haemoperfused Organs Research Group, Department of Comparative Medicine and Facilities of Experimental Animal Sciences, and of the Department of Laboratory Medicine, Charité, Humboldt University Berlin. We are grateful to Dr H. Hartmann, Institute of Veterinary Physiology of Freie Universität Berlin, for his support of the project, and to Alexandra Fischer for her committed technical assistance. All research contributing to this article has been carried out at the Institute of Veterinary Physiology and at the Department of Comparative Medicine and Experimental Animal Sciences. The corresponding author changed his position just recently. Schering AG has not been involved in any of the work presented in this paper.

Conflict of interest statement. None declared.

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Received for publication: 11.3.02
Accepted in revised form: 31.1.03