Evidence for protective roles of polyethylene glycol plus high sodium solution and trimetazidine against consequences of renal medulla ischaemia during cold preservation and reperfusion in a pig kidney model

Jean Pierre Faure1, Christophe Jayle1, Delphine Dutheil1, Michel Eugene1, Keqiang Zhang1, Jean Michel Goujon1, Isabelle Petit-Paris1, Jean Paul Tillement2, Guy Touchard1, Rene Robert1, Anne Wahl1, Francois Seguin1, Gerard Mauco1, Alain Vandewalle3 and Thierry Hauet1

1INSERM ERM Poitiers, Unité de Transplantation Expérimentale, Département de Génétique Animale, Institut National de Recherche Agronomique, Domaine du Magneraud, BP 52, 17700 Surgères, and Centre Hospitalier et Universitaire, Hôpital Jean Bernard, Poitiers, 2Laboratoire de Pharmacologie and Centre National de la Recherche Scientifique, Faculté de Médecine de Paris XII, F-94010 Créteil and 3INSERM U 478, IFR 02, Faculté de Médecine Xavier Bichat, Paris, France

Abstract

Background. The renal medulla is particularly sensitive to oxidant stress and to ischaemia–reperfusion injury (IRI). In organ transplantation, delayed graft function is an important problem and cold ischaemia is thought to be the most important factor in short- and long-term complications. Our aim was to study cold-induced damage in proximal tubular segments and renal medulla osmolite excretion during use of various preservation solutions, and to clarify the role of trimetazidine (TMZ) in limiting renal dysfunction.

Methods. Using an autotransplanted pig kidney model, we assessed renal tubule function, medullary osmolite excretion and renal damage between day 1 and week 2 after 24 or 48 h cold storage in University of Wisconsin solution (UW), Celsior and ECPEG (two new high Na+ preservation solutions) or the Hospital Edouard Herriot solution (HEH; a high Na+ version of UW). In additional groups, TMZ was added to these preservation solutions for 24 and 48 h cold storage.

Results. Renal function was reduced under these preservation conditions. Tubular injury was associated with aminoaciduria and with a limited Na+ reabsorption. Medullary damage led to the early appearance of trimethylamine-N-oxide and dimethylamine in urine. However, renal damage was modulated by preservation conditions. In addition, TMZ added to each of the solutions efficiently protected against IRI even after prolonged preservation.

Conclusion. TMZ efficiently protected kidneys against damage when added to the HEH and particularly ECPEG solutions, even after 24 h cold storage. These findings point to a role for drugs that target mitochondria, and demonstrate that TMZ may provide a valuable therapeutic tool against IRI and could be included in therapeutic protocols.

Keywords: cold preservation; ischaemia–reperfusion injury; pig kidney model; trimetazidine

Introduction

Ischaemic acute renal failure results in the permanent loss of peritubular capillaries and predisposes towards progression of chronic renal failure. Because of this, 50% of renal allografts no longer function after ~12 years. Ischaemia–reperfusion injury (IRI) is a perioperative event which causes further damage as well as a complex combination of insults involving multiple pathophysiological mechanisms. Calcium dyshomoestasis, oxygen free radical formation, mitochondrial dysfunction, cytokine generation and neutrophil sequestration/activation have all been identified as mediators of IRI [1]. Among these mediators, there is increasing evidence that mitochondria play a role in this process because of the profound changes that occur during ischaemia and reperfusion. Recent work,
examining both human renal tubular cells in culture and transplants in the clinical setting, indicates that cold temperatures cause necrosis while rewarming causes apoptosis [2]. This apoptosis has been shown to occur through a mitochondrial pathway and involves cold-induced mitochondrial permeability transition pore opening, which in turn produces mitochondrial swelling, mitochondrial membrane rupture and cytochrome c translocation to the cytosol [3]. Consequently, in organ transplantation, mitochondrial production of ATP regulation of ionic homeostasis and production of reactive oxygen species (ROS) point to a central role for these organelles in IRI.

In addition to acute events, recent studies have demonstrated detrimental impacts of prolonged ischaemia [4]. Using current preservation methods [Euro-Collins (EC), University of Wisconsin (UW)], we demonstrated that trimetazidine (TMZ) reduces cold ischaemia injury in kidneys preserved for 48 h [5]. TMZ is a derivative of pipermane, which is used for the treatment of angina pectoris. The anti-ischaemic property of TMZ is thought to be due to its ability to limit intracellular acidosis and to cause depletion of high-energy phosphates and accumulation of calcium, which are also major factors involved in IRI pathophysiology. Recently, several studies demonstrated that TMZ affords renal protection after hind limb ischaemia reperfusion and glycerol-induced acute renal failure [6]. UW continues to be considered the gold standard by which new cold storage preservation solutions are evaluated. However, clinical and experimental data demonstrate that this solution is not completely successful in preventing cold ischaemic graft injury. There are new concepts emerging in the area of preservation solutions. Celsior (CEL) solutions (using a high Na⁺ and low K⁺ ratio) and ECPEG solutions (polyethylene glycol combined with high Na⁺) were developed recently for multi-organ preservation [7]. A high Na⁺–low K⁺ version of UW has also been evaluated in different preservation conditions [8]. The present study was designed to identify the effects of cold ischaemia duration (24 and 48 h) and different preservation solutions with or without TMZ on renal function and damage.

**Materials and methods**

**Surgical procedures and preservation solution**

We used an established autotransplanted pig kidney model of cold IRI injury [9]. Briefly, following nephrectomy, kidneys were immediately cold flushed and preserved at 4°C for 24 or 48 h; the organs were then autotransplanted and contralateral nephrectomies were performed. The preservation solutions included UW, a low K⁺ version of UW (Hôpital Edouard Herriot solution, HEH) and newer low K⁺ CEL and ECPEG solutions. The animals were divided into 18 groups that included control and uninephrectomized (Nef) age-matched groups (n = 6 for both), and groups with 24 h UW preservation (UW24h, n = 10), 48 h UW preservation (UW48h, n = 8), 24 h CEL preservation (CEL24h, n = 10), 24 h HEH preservation (HEH24h, n = 10), 48 h HEH preservation (HEH48h, n = 8), 24 h ECPEG preservation (ECPEG24h, n = 10) and 48 h ECPEG preservation (ECPEG48h, n = 8). In additional groups, TMZ (10⁻⁹ M/l) was added to UW, CEL, HEH and ECPEG solutions for 24 and 48 h (n = 10 and 8, respectively). The preservation solutions are described further in Table 1. This study was performed in accordance with the Guidelines of the French Agricultural Office and the legislation governing animal studies.

**Renal function**

Pigs were placed in metabolic cages for 24 h in order to collect urine and blood samples. Endogenous creatinine clearance (CCr; ml/min) and fractional excretion of sodium (FENa; %) were measured before kidney preservation and on post-operative days 1, 3, 5, 7, 11 and 14 (D1–D14). CCr and FENa were calculated as previously described [14]. Blood and urine parameters were measured with an automatic analyser (Hitachi, Paris, France).

**NMR experiments**

As previously described, we collected urine and plasma samples from control and preserved kidneys. The samples were stored at -20°C until nuclear magnetic resonance measurements were performed [5]. For urine spectra, we calculated the ratio of trimethylamine-N-oxide (TMAO) in mmol/mol of creatinine. Oxidative metabolism was assessed from the ratio of citrate in urine. TMAO was also determined in plasma spectra (TMAOp) and was recorded only when its signal was intense enough to be separated from glucose resonances.
Histological studies

Kidney fragments obtained by ultrasonography-guided biopsy performed at various times after surgery were processed for light microscopy. Biopsy samples from the deep cortex–outer medulla region of the kidney were fixed in Dubosq-Brazil and 10% formalin in phosphate-buffered saline (PBS), embedded in paraffin, and stained with haematoxylin and eosin and periodic acid–Schiff (PAS). Two basic morphological patterns typical of proximal tubule cell lesions affecting a certain percentage of surface area were evaluated on five different PAS-stained tissue sections using a semi-quantitative graded scale: 0, none; 1, < 10%; 2, 10–25%; 3, 50–75%; and 4, > 75% [9]. The degree of mitochondrial injury was also determined using transmission electron microscopy (30 min to 1 h after reperfusion at day 7 and at 2 weeks after reperfusion) as previously described [9]. Mitochondrial injury, characterized by mitochondrial swelling, rupture of the inner and outer membranes, and leakage of mitochondrial matrix into cytoplasm, was examined at high magnification (×1000), using a semi-quantitative graded scale 0–4 as above.

Immunohistochemical studies

Frozen and paraffin-embedded kidney biopsy sections (5 μm) were processed for indirect immunohistochemistry using several mouse monoclonal antibodies (dilution 1:20). Sections were deparaffinized, rehydrated, and heated in a pressure cooker containing citrate buffer pH 6 to boiling point for 2 min. The sections were then cooled, rinsed in PBS and processed for indirect immunohistochemistry using the antibodies listed in Table 2 as previously described [9]. All sections were examined under blind conditions and were photographed. The number of MCA1218- and CD4⁺-labelled cells per surface area (10⁴/μm²) was counted on five different tissue sections for each of the experimental conditions as previously described [9].

Statistical analysis

Mean values were calculated for each group (mean ± SEM) and differences were compared by repeated measures ANOVA using the Instat (version 2.04) software package from GraphPad (San Diego, CA). Unpaired t-tests were used for cellular infiltration, and Mann–Whitney U-tests were used for histological data analyses and immunohistochemical data. The Mann–Whitney U-test was also used to investigate whether spectroscopic characteristics differed between kidneys showing immediate post-transplant function and those with acute tubular necrosis. Differences with P-values < 0.05 were considered significant.

Results

Effect of cold ischaemia time and TMZ on renal function and survival

Total body weights and kidneys weight were not different between control Nef and experimental groups (data not shown). Three pigs died on post-operative days 6 and 10 in groups UW48h and CEL48h due to development of primary non-functioning kidneys. Survival was 100% in the control group and in all remaining groups. Functional data were not determined in groups UW48h and CEL48h (< 100 ml/24 h) because of a prolonged anuria before D3 and D4, respectively. As shown in Figure 1, cold ischaemia and reperfusion affected renal function after autotransplantation and correlated with time of preservation. The highest C_{Cr} values were in experimental groups HEH and especially ECPEG, and TMZ improved renal function in all preserved groups particularly when combined with ECPEG (Figure 1A). Tubular function, determined by FENa, was also improved in the TMZ groups and especially when combined with ECPEG (Figure 1B and C).

Effect of cold ischaemia and TMZ on medulla injury, oxidative metabolism and mitochondrial integrity

Citrate excretion was significantly improved between D1 and W2 in all experimental groups preserved with TMZ (Figure 2A). Table 3 summarizes the degrees of mitochondrial alterations. ECPEG with TMZ was the more efficient combination for reducing mitochondrial injury after 24 h and particularly 48 h of cold ischaemia. TMAO excretion in urine was more intense in the UW and CEL groups and significantly reduced in the HEH and, in particular, the ECPEG groups. TMZ significantly reduced TMAO excretion in all experimental groups (Figure 2B and C). TMAOp was detected in the UW and CEL groups between D1 and D14 and was not detected after D7 in the HEH and ECPEG groups. TMZ significantly reduced TMAOp in all experimental groups (Figure 2D).

Effect of cold ischaemia and TMZ on preservation of graft morphological features

Table 4 and Figure 3 summarize the degree of cellular damage to proximal tubule cells from the reperfused kidneys. There were marked tubule cell lesions in autotransplanted kidneys from the UW and CEL.
Fig. 1. Effect of cold ischaemia and TMZ on glomerular filtration rate (GFR; ml/min) (A), sodium excretion (B) and urine concentration (C). Renal function was also determined in control and uninephrectomized animals (control, ●; Nef, ○). Autotransplanted kidneys were cold flushed and preserved with University of Wisconsin solution for 24 h (UW24h) and 48 h (UW48h), CEL for 24 h (CEL24h) and 48 h (CEL48h), HEH for 24 h (HEH24h) and 48 h (HEH48h) and ECPEG for 24 h (ECPEG24h) and 48 h (ECPEG48h) or with the same preservation solutions plus TMZ (*P < 0.05 CEL and UW vs HEH and ECPEG; CEL vs CELTMZ, UW vs UWTMZ, ECPEG vs ECPEGTMZ or HEH vs HEHTMZ; **P < 0.01 CEL and UW vs HEH and ECPEG; CEL vs CELTMZ, UW vs UWTMZ, ECPEG vs ECPEGTMZ or HEH vs HEHTMZ).
Fig. 2. Effect of cold ischaemia and TMZ on citrate excretion (A), TMAO excretion (B) and TMAOp (C). Autotransplanted kidneys were cold flushed and preserved with University of Wisconsin solution for 24 h (UW24h) and 48 h (UW48h), CEL for 24 h (CEL24h) and 48 h (CEL48h), HEH for 24 h (HEH24h) and 48 h (HEH48h) and ECPEG for 24 h (ECPEG24h) and 48 h (ECPEG48h) or with the same preservation solutions plus TMZ (*P < 0.05 CEL and UW vs HEH and ECPEG, CEL vs CELTMZ, UW vs UWTMZ, ECPEG vs ECPEGTMZ or HEH vs HEHTMZ; **P < 0.01 CEL and UW vs HEH and ECPEG, CEL vs CELTMZ, UW vs UWTMZ, ECPEG vs ECPEGTMZ or HEH vs HEHTMZ).
groups. The HEH and particularly the ECPEG preservation solutions were the more efficient in reducing renal damage after 24 and 48 h of preservation (Table 4). TMZ efficiently limited tissue damage in all experimental groups and particularly in combination with ECPEG.

**Effect of cold ischaemia and TMZ on CD4⁺, monocyte and macrophage infiltration**

HEH and ECPEG efficiently reduced CD4⁺ cell infiltration. In addition, kidneys preserved in UW and CEL exhibited a reduction in CD4⁺ cell infiltration when TMZ was added to the preservation solution (Figure 4A). A stronger beneficial effect of TMZ was observed when it was added to ECPEG, particularly after 48 h of preservation. Positive staining with the MC1218 macrophage/monocyte marker was detected in all kidney biopsies taken 5 days after transplantation (Figure 4B). However, there were more MC1218-positive cells in post-transplanted kidneys from groups CEL and UW after 24 and 48 h of preservation. These macrophage/monocyte infiltrations were reduced in biopsy samples taken 2 weeks after transplantation from 24 and 48 h cold-stored kidneys. In contrast, MC1218-positive cells were detected on biopsy samples taken 12 weeks following transplantation. The number of MCA1218-positive cells was much lower in cold-flush kidneys that were preserved for 24 and 48 h in UW and CEL solutions with TMZ than in kidneys preserved with UW and CEL without TMZ. TMZ also efficiently improved CEL preservation efficiency after 72 h of preservation when compared with CEL alone.

**Discussion**

The mechanisms underlying the detrimental effects of cold ischaemia and thermal injury on graft survival remain unclear and the factors that trigger and control the repair process are poorly understood. The renal medulla and in particular the outer medulla is a prominent target during ischaemia reperfusion [11]. In several studies, five major factors have been correlated repeatedly with reduced graft function and survival: donor age, brain death, preservation and reperfusion injury, immune injury, and rejection and recipient

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### Table 3. Quantification of mitochondrial injury in early (30 min to 1 h) and day 7 post-transplanted pig kidneys that were evaluated and given semi-quantitative scores (see Materials and methods)

<table>
<thead>
<tr>
<th>Time</th>
<th>Groups</th>
<th>UW/UWTMZ</th>
<th>CEL/CELTMZ</th>
<th>HEH/HEHTMZ</th>
<th>ECPEG/ECPEGTMZ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>24 h cold storage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min–1 h</td>
<td>Cell detachment</td>
<td>2.2 ± 0.1*(#)/1.7 ± 0.1*</td>
<td>2.3 ± 0.1*(#)/1.8 ± 0.1*</td>
<td>1.8 ± 0.1*(#)/1.4 ± 0.1*</td>
<td>1.0 ± 0.1*(#)/0.9 ± 0.1*</td>
</tr>
<tr>
<td>Day 7</td>
<td></td>
<td>1.6 ± 0.1*(#)/1.2 ± 0.1*</td>
<td>1.7 ± 0.1*(#)/1.2 ± 0.1*</td>
<td>1.2 ± 0.1*(#)/0.8 ± 0.2*</td>
<td>0.7 ± 0.1*(#)/0.4 ± 0.1*</td>
</tr>
<tr>
<td><strong>48 h cold storage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min–1 h</td>
<td>Cell detachment</td>
<td>3.0 ± 0.1*(#)/2.4 ± 0.2*</td>
<td>3.1 ± 0.1*(#)/2.7 ± 0.1*</td>
<td>2.4 ± 0.2*(#)/1.9 ± 0.1*</td>
<td>1.2 ± 0.1*(#)/0.8 ± 0.1*</td>
</tr>
<tr>
<td>Day 7</td>
<td>Tubular dilatation</td>
<td>2.5 ± 0.1*(#)/1.9 ± 0.2*</td>
<td>2.6 ± 0.1*(#)/2.1 ± 0.1*</td>
<td>1.8 ± 0.2*(#)/1.3 ± 0.1*</td>
<td>1.0 ± 0.1*(#)/0.8 ± 0.2*</td>
</tr>
</tbody>
</table>

*\(\#\)P < 0.05 CEL and UW vs ECPEG and HEH or CELTMZ and UW/CEL vs HEHTMZ and ECPEG/ECPEGTMZ; \(\#\)P < 0.05 CEL vs CELTMZ, UW vs UW/CEL or HEH vs HEHTMZ, ECPEG vs ECPEG/ECPEGTMZ; \(\#\)P < 0.05 HEH or HEHTMZ vs ECPEG or ECPEG/ECPEGTMZ.

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### Table 4. Degree of proximal tubule cell lesions from early (30 min to 1 h) and day 7 post-transplanted pig kidneys

<table>
<thead>
<tr>
<th>Injury type</th>
<th>Time</th>
<th>Groups</th>
<th>UW/UWTMZ</th>
<th>CEL/CELTMZ</th>
<th>HEH/HEHTMZ</th>
<th>ECPEG/ECPEGTMZ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell detachment</strong></td>
<td>30 min–1 h</td>
<td></td>
<td>2.2 ± 0.1*(#)/1.8 ± 0.1*</td>
<td>2.3 ± 0.1*(#)/1.9 ± 0.1*</td>
<td>1.5 ± 0.1*(#)/0.9 ± 0.1*</td>
<td>1.0 ± 0.1*(#)/0.5 ± 0.1*</td>
</tr>
<tr>
<td>Day 7</td>
<td></td>
<td></td>
<td>1.6 ± 0.1*(#)/1.2 ± 0.1*</td>
<td>1.6 ± 0.1*(#)/1.2 ± 0.1*</td>
<td>1.1 ± 0.1*(#)/0.6 ± 0.2</td>
<td>0.5 ± 0.1*(#)/0.2 ± 0.2</td>
</tr>
<tr>
<td><strong>Tubular dilatation</strong></td>
<td>30 min–1 h</td>
<td></td>
<td>2.6 ± 0.1*(#)/2.0 ± 0.1*</td>
<td>2.5 ± 0.1*(#)/2.0 ± 0.1*</td>
<td>1.5 ± 0.1*(#)/0.6 ± 0.1</td>
<td>0.6 ± 0.1*(#)/0.2 ± 0.1</td>
</tr>
<tr>
<td>Day 7</td>
<td></td>
<td></td>
<td>1.6 ± 0.1*(#)/1.1 ± 0.1*</td>
<td>1.6 ± 0.1*(#)/1.2 ± 0.1*</td>
<td>1.0 ± 0.1*(#)/0.6 ± 0.1</td>
<td>0.5 ± 0.1* (#)</td>
</tr>
</tbody>
</table>

**24 h cold storage**

*\(\#\)P < 0.05 CEL and UW vs ECPEG and HEH or CELTMZ and UW/CEL vs HEHTMZ and ECPEG/ECPEGTMZ; \(\#\)P < 0.05 CEL vs CELTMZ, UW vs UW/CEL or HEH vs HEHTMZ, ECPEG vs ECPEG/ECPEGTMZ; \(\#\)P < 0.05 HEH or HEHTMZ vs ECPEG or ECPEG/ECPEGTMZ.

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factors. Interestingly, four of these are non-immune, and could be addressed by donor selection and organ allocation strategies, by strategies to reduce non-immune injury and stress, or both methods [12].

The first major result of the present study was that cold ischaemia and particularly long preservation time (48 h) induced further functional deterioration compared with 24 h of preservation. These functional results were related to tissue damage, which was more important in the UW and CEL than in the HEH and ECPEG groups. The HEH- and particularly the ECPEG-preserved groups exhibited more efficient renal function. We also demonstrated that TMZ improved renal function after cold preservation, particularly in the ECPEG group. Therefore, our data support previous findings showing that high Na\(^+\) preservation solutions are efficient in diminishing renal dysfunction. In addition, we demonstrated a role for colloids in renal preservation and found a particularly strong effect with PEG.

In addition to the extensive adverse functional changes caused by cold IRI, we also found that PEG combined with TMZ was particularly efficient in reducing renal medulla injury as determined by TMAO. Water-stressed cells accumulate small organic molecules to maintain cellular osmotic balance and cell morphology. These small osmolytes generally include amino acids and their derivatives, carbohydrates and methylamines. The methylamines are referred to as ‘counteracting osmolytes’ because they can reverse the perturbations caused by urea [13]. A key paradigm in the biology of adaptation holds that urea affects protein function by increasing the fluctuations of the native state, while TMAO affects function in the

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**Fig. 3.** Effect of cold ischaemia and TMZ on degree of proximal tubular cell lesions at day 14. Autotransplanted kidneys were cold flushed and preserved with University of Wisconsin solution for 24 h (UW24h) and 48 h (UW48h), CEL for 24 h (CEL24h) and 48 h (CEL48h), HEH for 24 h (HEH24h) and 48 h (HEH48h) and ECPEG for 24 h (ECPEG24h) and 48 h (ECPEG48h) or with the same preservation solutions plus TMZ.
opposite direction by decreasing the normal fluctuations of the native ensemble. TMAO thus counteracts the damaging effects of concentrated urea on protein folding and stability [14]. Such modifications are likely to play a role in cell dysfunction, especially in epithelial cells whose optimal function depends on the polarity in membrane fluidity between the apical and basolateral domains. We demonstrated that urinary TMAO excretion was strongly reduced in the HEH and especially in the ECPEG groups. In addition, TMZ reduced TMAO excretion in all experimental groups. Previous studies have demonstrated that early appearance of TMAO in urine is related to medullary damage (for a review, see [15]). Consequently, our data demonstrated greater renal medulla protection with HEH and ECPEG and when TMZ was added in the preservation solutions. The intensity of TMAOp is correlated with the degree of renal failure determined by creatinine. TMAO is efficiently cleared by well-preserved kidneys and its levels are related to the functional level of the kidneys. Consequently, the present data indicate that an important component of ischaemic acute renal failure pathophysiology is related to reductions in medullary blood flow along with reductions in oxygen delivery to tubular structures. TMAO excretion is a marker of renal medulla integrity and is correlated with renal function. Recent studies have focused on the positive effect of TMAO on protein folding, which is apparently mediated via a solvophobic mechanism [13]. Consequently, we suggest that TMAO excretion may be related to renal medulla integrity, and that preservation of this natural osmolyte in renal medulla may be related to a beneficial influence on renal function via its role in protein folding efficiency.

The formation of citrate from acetyl coenzyme A and oxaloacetate is catalysed by citrate synthase with...
coenzyme A-SH as a byproduct. Our data demonstrated that citrate excretion was increased in the HEH and particularly in the ECPEG groups. In addition, the citrate excretion showed that TMZ improved the preservation solutions, particularly in combination with the ECPEG solution. In previous reports, reduced citrate excretion in urine was correlated with impaired oxidative metabolism particularly during proximal tubular injury [15]. Citrate excretion was also correlated with aminoaciduria, which was demonstrated by alanine excretion. These markers, which are associated with TMAO excretion, are strongly related to evidence of medullary injury [15]. Consequently, the antioxidant activity of TMZ and its protective effect on ATP levels may protect both cell membrane and endothelial integrity from IRI, particularly when combined with ECPEG. We hypothesize that increased citrate synthase activity results in increased ATP production by efficiently preserving the oxidative phosphorylation process. The urinary excretion of citrate is independent of plasma citrate levels. Both intracellular pH and the intracellular bicarbonate concentration control the rate of citrate transport across the inner mitochondrial membranes as well as the activity of the citric acid cycle and the degree of citrate utilization by renal tubular cells [16]. In addition, because the filtered load of citrate remains relatively constant, proximal tubular citrate reabsorption regulates urinary citrate excretion. In our study, Na\(^+\) reabsorption was significantly increased in the TMZ- and ECPEG-preserved groups. Consequently, the higher urinary citrate was probably due in part to maturational differences in the proximal tubule and not simply to Na\(^+\)/citrate co-transport or mitochondrial function. Collectively, citrate and TMAO are discriminant markers that assess organ preservation quality.

Furthermore, the present data indicate that cellular infiltration strongly correlates with the intensity of renal dysfunction and damage, which was significantly reduced in implanted kidneys preserved for 24 h compared with those preserved for 48 h. Our data also suggest that T cells play a major role in the development of renal IRI, an effect that is probably mediated by adhesion of infiltrating T cells to renal tubular cells. While using a pig kidney autotransplant model, we recently demonstrated a role for 48 h cold storage and for T cells as pathogenic factors in ischaemic injury [17]. Classically, T-cell activation has been thought to require foreign antigen bound to a self-major histocompatibility complex (MHC) molecule together with co-stimulatory signals by antigen-presenting cells. The absence of foreign antigens in this autotransplant model suggests that alloantigen-independent T-cell activation may be involved in renal IRI. An antigen-independent mechanism of T-cell activation has been described that involves chemokines (for a review, see [18]). Oxygen free radicals, generated during IRI, can also activate T cells. Our data are strongly supported by recent reports which demonstrated that T cells, and more particularly CD4\(^+\) T cells, are important mediators of ischaemic injury [24]. We also demonstrated a role for TMZ in the inflammatory process of IRI. Kidneys from TMZ-preserved groups exhibited reduced cell infiltration. HEH and particularly ECPEG plus TMZ very efficiently reduced one of the major negative effects of IRI that can influence the outcome of the grafts.

This study demonstrated an important role for CD4\(^+\) T cells in renal IRI and a possible modulation of IRI by preservation conditions. In addition, TMZ efficiently protected kidneys from IRI when added to different preservation solutions. TMZ recently was demonstrated to have beneficial effects against cyclosporin-induced nephrotoxicity, a condition where ROS play a pivotal role [19]. We further demonstrated a potential role for extracellular solutions. In the clinical setting, intracellular-type solutions are the most widely used for organ preservation, but extracellular solutions may also improve organ preservation. Our data point to a role for colloid and particularly PEG, a role that is more important when combined with high Na\(^+\) solutions.

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Conflict of interest statement. None declared.

References


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