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Lidocaine-Containing Euro-Colins Solution Prevents Renal Injury in the Isolated Perfused Canine Kidney Exposed to Prolonged Cold Ischemia

N. ERKASAP, E. ATES[1], S. ERKASAP[1], Z. KAYGISIZ

Department of Physiology and [1] Department of General Surgery, Osmangazi University, Medical Faculty, Eskisehir, Turkey

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Summary

Previous studies have reported a decreased incidence of delayed graft function after cadaveric transplantation with the use of lidocaine pretreatment of the donor. We evaluated the effects of lidocaine on prolonged cold ischemia and reperfusion injury in a canine model of isolated kidney perfusion (IPK). The purpose of this study was to evaluate the renal function of isolated perfused canine kidneys after 48 h of cold storage with Euro-Collins (EC) solution or EC solution plus lidocaine. Isolated perfused canine kidneys were randomized into four groups which contained six kidneys: I) cold flush with EC solution and immediately reperfused, II) cold flush with EC solution plus lidocaine and immediately reperfused, III) 48 h of cold storage with EC and reperfusion, IV) 48 h of cold storage with EC solution plus lidocaine and reperfusion. The measured renal functions were glomerular filtration rate, urine production, perfusate flow, urinary lactic dehydrogenase (ULDH), Na reabsorptive capacity, and tissue MDA levels. Histological examination was performed after reperfusion. The tubular functions of kidneys preserved with EC solution containing lidocaine were better when compared with the kidneys preserved with EC alone. Tubular injury marker levels (ULDH) in group IV were significantly lower than in group III and lidocaine also reduced lipid peroxidation during reperfusion. This is in agreement with the histological results. The results of the present study can be taken as evidence of the cytoprotective effect of lidocaine, which may therefore be accepted as a useful agent for kidney preservation.

Key words

Cold-storage • Lidocaine • Kidney • Isolated kidney perfusion

Introduction

Hypothermic storage following initial flush perfusion remains the most widely used technique for preserving cadaveric renal allografts. Organs stored hypothermically for transplantation show varying degrees of tissue injury, depending on the duration of preservation (Ploeg *et al.* 1988). Ischemic injury to renal allografts before implantation is an important cause of delayed graft function (Hauet *et al.* 1997). However, the causes of this injury are not entirely clear. In the transplanted grafts, marked prominent tissue injury appears during the reperfusion period following a cold or a warm period of ischemia.

The pathogenesis of this ischemia-reperfusion (I/R) injury is complex. Putative mechanisms include the formation of oxygen-derived free radicals (McCord 1985), an absence of the control of calcium metabolism in the cell (Farber *et al.* 1981), depletion of adenosine triphosphate (Linas *et al.* 1990, Trump and Berezesky 1995), the obstruction of capillaries by various preformed blood elements, and a consequent decreased blood flow (Leaf 1973).

Kidney preservation can be improved in two major ways: 1) by limiting cold ischemia injury during cold storage, 2) by protecting organs from the damage caused by the initial reperfusion (Bonventre and Weinberg 1992).

The local anesthetic lidocaine has been shown to be protective against myocardial I/R injury, although its mechanism of action remains unresolved (Homeister *et al.* 1990). Furthermore, lidocaine has been shown to be effective against postischemic reperfusion injury in isolated rat lungs (Das and Misra 1994). The effect of lidocaine on the ischemic normothermic rat heart was studied in a Langerdorff preparation. Cardiac arrest with lidocaine thus reduced lactate formation during ischemia and attenuated high-energy phosphate depletion after reperfusion (Bengtsson *et al.* 1991). Lidocaine has been reported to decrease the incidence of delayed graft function after transplantation by lidocaine pretreatment of the donor (Walaszewski *et al.* 1988, 1991).

We evaluated the effect of lidocaine on prolonged cold ischemia and reperfusion injury in a canine model of isolated kidney perfusion (IPK). The IPK model was selected because it demonstrates a reproducible degree of renal damage after preservation, which is not influenced by systemic effects and provides simple control of pressure and flow.

Methods

Experiments were carried out using 12 adult male conditioned mongrel dogs weighing 20 to 25 kg. Twelve hours before the experiments, dogs were fasted but allowed free access to water.

General anesthesia was induced by i.v. injection of 30 mg/kg dose of phenobarbital sodium, with supplemental small doses given as needed. Dogs were intubated, and ventilated with a respirator at the rate of 20 cycles/min with a tidal volume of 20 ml/kg body weight.

Animals were given 600 ml 0.9 % saline i.v. during the surgical procedure. Both kidneys were exposed through a midline abdominal incision and freed from perirenal tissue and fat. The kidneys were flushed immediately after nephrectomy *via* an arterial cannula with 200-250 ml of the cold preservation solution (4 ^{o}C), which was held 100 cm above the arterial cannula (flush out time 1-3 min).

The kidneys were randomized into four groups, each containing six kidneys:

***Group I*:** (n=6) Immediately perfused after cold flush with Euro-Collins (EC) solution.

***Group II*:** (n=6) Immediately perfused after cold flush with EC solution containing lidocaine hydrochloride (20 μ/ml) (Tems-Biosel, B6a).

***Group III*:** (n=6) Perfusion after 48 h of cold storage with EC following cold flush.

***Group IV*:** (n=6) Perfusion after 48 h of cold storage with EC containing lidocaine (20μ/ml) following cold flushing.

Groups I and II served as the control groups for experimental groups III and IV. The concentration of lidocaine was similar to that used by Schmid *et al.* (1996).

Perfusion of kidneys was then performed *via* the renal artery with freshly prepared Krebs solution at 37.5 ^{o}C containing 10 mg/l creatinine (Sigma) as described previously (Türker *et al.* 1988). Perfusion was carried out by a Multifix peristaltic pump (Betriebsart, DB; Schutzart: IP21, Germany). Perfusion pressure (PP) was measured with a pressure transducer (Statham P23 Dc., Grass, Quincy, MA, USA). The pH and O_2 tension were maintained with a membrane oxygenator (Midflo D705, Dideco hallow fiber oxygenator, Italy) purged with $O_2 : CO_2$ (95 % : 5 %). The pH was 7.38-7.42 in the prewarmed medium and did not change during reperfusion. Renal perfusion flow rate (PFR) was adjusted to maintain mean arterial perfusion pressure at 100-120 mm Hg. After the equilibration period lasting 30 min, the following parameters were evaluated every 10 min: Perfusion flow rate (PFR) (ml/min/g) was measured by collecting the perfusate every 10 min. Glomerular filtration rate (GFR) (μl/min/g) was evaluated from creatinine clearance. Urine was collected into tubes and urine flow rate (UFR) (μl/min/g) was determined. Fractional sodium reabsorption (%Na) were calculated by standard procedures. Urinary lactic dehydrogenase (ULDH) (U/l) was also monitored.

Analytical methods

Sodium, creatinine, and urine LDH levels were measured with an automatic analyzer (Hitachi 911, Germany). Oxygen partial pressure was assessed by using

a gas analyzer (Radiometer Copenhagen, ABL 555, Denmark).

At the end of isolated perfusion, the susceptibility of the kidney to lipid peroxidation was determined by evaluating malondialdehyde (MDA) levels, which were measured by thiobarbituric acid test described by Uchiyama and Mihara (1978). Briefly, 3 ml 1 % phosphoric acid and 1 ml 0.6 % thiobarbituric acid were added to 0.5 ml homogenate and the mixture was kept in a water bath at 95 °C for 45 min. The colored reaction product was extracted with 4 ml n-butonal, and the difference of the absorbance at 535 and 520 nmol was recorded. The results were expressed as nanomoles MDA per gram tissue (nmol/g).

Histological examination

At the end of isolated perfusion, kidney samples were taken for light microscopy and fixed with 10 % formalin then embedded in paraffin and stained with hematoxylin-eosin (periodic acid-Schiff). The light microscopic study was reviewed by a pathologist uninformed about the experimental conditions. Light microscopic sections were examined for cell vacuolization, tubular dilatation, interstitial edema, intracellular edema, tubular cell brush border integrity, tubular necrosis, and cell detachment. Histological lesions were graded by using a semi quantitative scale: 1 - no abnormality, 2 - mild lesions, affecting 10 % of kidney samples, 3 - lesions affecting 25 % of kidney samples, 4 - lesions affecting 50 % of kidney samples, 5 - lesions affecting more than 75 % of kidney samples.

Statistical analysis

Results were expressed as means ± S.D. and compared using analysis of variance and the Mann-Whitney U test. $P<0.05$ value was considered statistically significant.

Results

GFR, PFR, UFR, %Na, urine LDH/GFR and tissue MDA levels were not significantly different between control groups I and II during reperfusion as shown in Figures 1 and 2.

GFR, mean PFR, UFR and fractional Na reabsorption were clearly decreased in groups III and IV following 48 h of cold storage when compared with groups I and II. Values of GFR in the kidneys preserved for 48 h with lidocaine added to EC were elevated compared to those preserved with EC alone ($p<0.05$) (Fig. 1A). After 48 h of cold storage, mean PFR values in the kidneys preserved with lidocaine added EC solution were higher than the mean value for the kidneys preserved in EC alone ($p<0.05$) (Fig. 1B). UFR in the kidneys preserved for 48 h with lidocaine added to EC were also increased compared to those preserved with EC alone ($p<0.01$) (Fig. 1C).

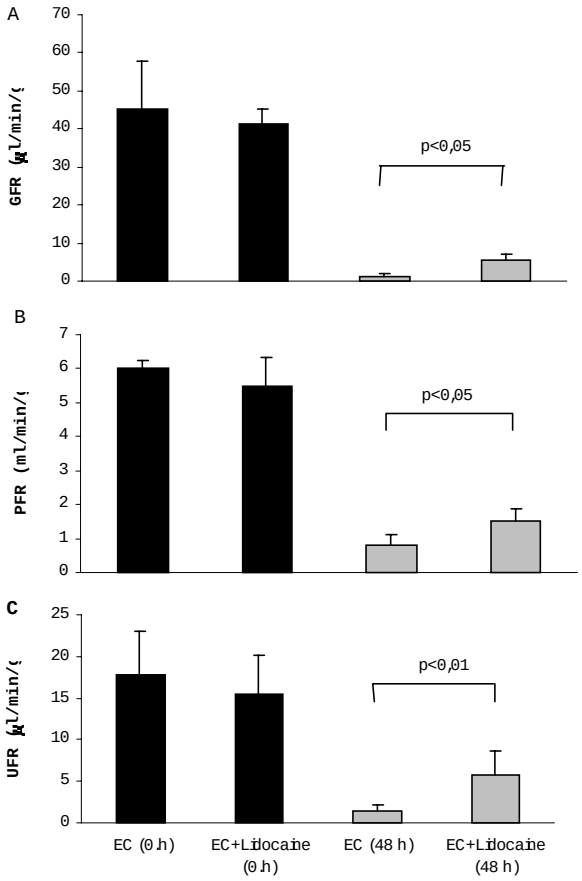

Fig. 1. *Effect of lidocaine on function of preserved canine kidneys. GFR, creatinine clearance; PFR, perfusate flow rate; UFR, urine flow rate. Values shown are the mean ± S.D.*

Lidocaine increased Na^+ reabsorption after 48 h of cold storage ($p<0.05$) (Fig. 2A). Lidocaine had a significant beneficial effect on urine LDH/GFR after 48 h of cold storage ($p<0,001$) (Fig. 2B). Renal MDA activity in the kidneys preserved with lidocaine added to EC solution was lower than the values in the EC group after 48 h of cold storage ($p<0.05$) (Fig. 2C).

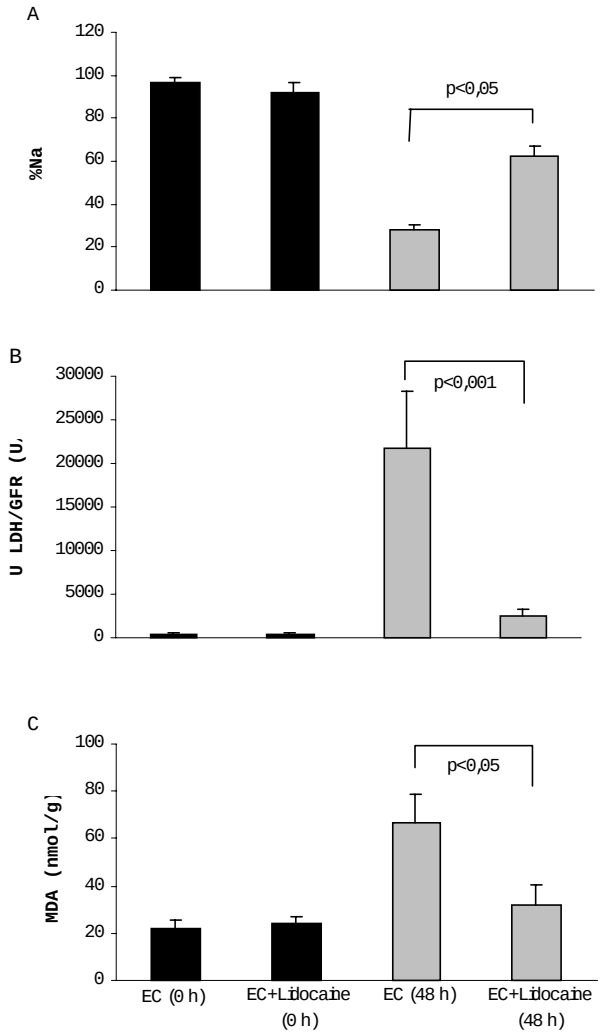

Fig. 2. *Effect of lidocaine on function and metabolic parameters of preserved canine kidneys. %Na, Fractional Na reabsorption in the urine; U LDH/GFR, Urine LDH / creatinine clearance; MDA, tissue levels for lipid peroxidation marker. The values shown are means ± S.D.*

After 48 h of cold storage, the tissue damage was significantly more expressed in the EC group ($p<0.001$) in both EC and lidocaine added EC groups. The results of this histological study are presented in Table 1.

Discussion

Kidneys stored in EC solution for longer than 30 hours often display delayed graft function after transplantation. Many investigators have tried to improve renal preservation by using a modified cold preservation solution, but there has been little advance in kidney preservation during the past 20 years (Ploeg *et al.* 1988).

Several mechanisms may participate in destroying cells during preservation of organs for transplantation. Cold ischemia stimulates glycolysis and glycogenolysis; it also increases the production of lactic acid and the concentration of hydrogen ions. Tissue acidosis can damage cells, induce lysosomal instability and alter mitochondrial properties (Rehncrona *et al.* 1979). A second mechanism concerns adenosine triphosphate (ATP), which is degraded during hypothermic storage, and this degradation results in the formation of end-products to which the plasma membrane is freely permeable (Belzer and Southard 1988). A third mechanism involves energy depletion, which leads to profound disturbances of electrolyte homeostasis with cellular loss of K^+ and accumulation of Na^+, Cl^-, Ca^{2+} and H^+ resulting in cell swelling, increased cytosolic Ca^{2+} and cytosolic pH changes (Trump and Berezesky 1995). Finally, reperfusion of injured tissue leads to the formation of O_2-derived free radicals and O_2 reactive species that cause a loss of organ viability (Parks *et al.* 1983, Ploeg *et al.* 1988).

The effects of lidocaine on nerve and muscle cell membranes have been extensively studied. This has been suggested as a beneficial agent for reducing the myocardial damage. The mode of action was attributed to its membrane stabilizing properties. However, Das and Misra (1992) reported the beneficial effect of lidocaine as a scavenger for toxic oxygen species and thereby reduced membrane lipid peroxidation. Thus the antioxidant property of this drug might, in part, be responsible for protecting the lungs against reperfusion injury (Das and Misra 1994). Lantos *et al.* (1996) also reported the scavenger action of lidocaine on free radical processes but they emphasized that lidocaine had no direct effect on polymorphonuclear leukocyte activation in the early phase of reperfusion following ischemia. Other studies reported that lidocaine reduced lung allograft reperfusion injury and inhibited PMN adhesion and subsequent migration to the lung allograft (Schmid *et al.* 1996). But the effect of lidocaine on free oxygen radical scavenger still remains to be explained on molecular level.

Tissue MDA activity is supposed to be an index of lipid peroxidation. Lipid-based cell membranes are primary targets of oxygen-derived radicals (Halliwell and Gutteridge 1989). The end products of lipid peroxidation include MDA, other aldehydes, hydrocarbon gases, and

conjugated dienes (Del Maestro 1980). We found that tissue MDA levels were lower in the kidneys preserved with lidocaine added EC solution, suggesting the diminished lipid peroxidation and tissue injury. This diminished tissue injury was also supported by the histopathological evaluation of kidney tissue specimens obtained 48 h after cold ischemia.

Table 1. Histopathological evaluation in kidneys of the groups.

Histology	**EC (0 h)**	**EC+Lidocaine (0 h)**	**EC (48 h)**	**EC+Lidocaine (48 h)**
Tubular necrosis	1.1	1.1	4.7	2.4
Tubular dilatation	1.3	1.3	4.6	2.0
Interstitial edema	1.3	1.5	4.7	2.4
Brush border	1.3	1.5	4.7	2.4
Cell detachment	1.2	1.5	4.6	2.4
Vacuolization	1.2	1.1	4.7	2.4
Interstitial cells	1.2	1.3	4.6	2.3
SCORE	1.25±0.1	1.3±0.11	4.6±1.25 *	2.3±0.12

** p<0.001 EC (48 h) vs EC + Lidocaine (48 h).*

Renal ischemia results in ATP depletion and ATP deficiency leads to disturbance in intracellular homeostasis of cytosolic Ca^{2+}, presumably by a combination of impaired extrusion and enhanced influx, so that cytosolic Ca^{2+} may increase during hypoxia in renal tubules (Peters *et al.* 1998). Changes in cytosolic Ca^{2+} and its relationship to loss of mitochondrial membrane potential and cell killing were characterized in single cells, whereas ATP and LDH release were determined in proximal tubular epithelium (Chi *et al.* 1995).

Isolated perfusion of the heart with Ca^{2+} free perfusate followed by Ca^{2+} containing perfusate causes dramatic alterations in the physiology and biochemistry of the tissue, a phenomenon known as the calcium paradox. A similar paradoxical effect of Ca^{2+} has also been reported to occur in the kidney (Duncan and Morton 1996). The mitochondria of the proximal tubule cells were swollen and subdivided with internal septa at the end of the calcium paradox (Morton *et al.* 1994). Isolated perfused kidney releases LDH in response to the artificial conditions of the calcium paradox (Morton *et al.* 1994, Duncan and Morton 1996).

Vital cells contain numerous enzymes that are necessary for the metabolism and maintenance of cellular function. Intracellular enzymes may be released in adverse conditions. Following ischemia, the enzyme lactate dehydrogenase (LDH) is released by kidney (Gebhard *et al.* 1977). Enzymes leave the kidney not only in the renal venous blood but also *via* urine. Glomerular filtration of LDH is not possible due to its molecular size, provided that the glomeruli are intact (Raab 1972). It is known that glomeruli are relatively resistant to ischemic injury. Hence, it is reasonable to assume that in our experiment the urinary LDH originates in the tubular cells. Kehrer *et al.* (1989) proposed that the LDH/GFR ratio is more suitable for assessment of the extent of ischemic stress than urinary LDH concentration alone. As we have shown in our experiments, decreased LDH/GFR values of kidneys preserved with the lidocaine added EC solution is not only due to increased GFR but also to lesser LDH release into the tubule.

Several studies reported that the prolonged cold ischemia decreased the PFR, GFR, UFR, and sodium reabsorption (Southard *et al.* 1985, Ploeg *et al.* 1988). The two control groups clearly differed in these parameters from two experimental groups in our study. Thus, renal functions of kidneys preserved with the lidocaine added EC solution were protected better than by EC alone. Walaszewski *et al.* (1988, 1991) reported that lidocaine administration to the donor significantly increased the incidence of immediate function of the kidneys after living and cadaveric transplantation. The occurrence of delayed graft function decreased from 67 % in lidocaine pretreated donors to 32 % in lidocaine non-pretreated donors in their study.

Our data which were obtained on an IPK model demonstrated that kidneys preserved with EC solution containing lidocaine exhibited better functional and histological parameters than kidneys preserved with EC alone after a long ischemia period. Our results further support the cytoprotective effect of lidocaine indicating that it may therefore be useful for kidney preservation against long-term cold ischemia.

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Reprint requests

Dr. Serdar Erkasap, Hasan Polatkan Bulvarı, Akın Sitesi, No: 122/13, Eskisehir, Turkey, fax : +90 222 2393772, e-mail: serkasap@ogu.edu.tr