Turk J Med Sci
34 (2004) 161-164

ORIGINAL ARTICLE

Effect of Vitamin E on Kidney Preservation Using Isolated Perfused Dog Kidney

Adnan ŞAHİN, Cem ALGIN, Çağatay SEZGİN, Enver İHTİYAR
Department of General Surgery, Faculty of Medicine, Osmangazi University, Eskisehir - Turkey

Received: December 25, 2003

Abstract: Unsuccessful cadaveric kidney transplantation still remains as an important problem in organ transplantation. Cold storage solutions are not very efficient for kidney preservation. Vitamin E is an important dietary antioxidant, which may play a vital role in preventing free radical perfusion injury. We investigated the effect of vitamin E on kidney preservation with Euro-Collins (EC) solution using the isolated perfused dog kidney model. Recipients were randomly divided into 3 groups: group 1 (n = 6), immediately reperfused with EC solution, given laboratory pelleted diet and drinking water for 4 weeks; group 2 (n = 6), flushed, and stored with EC solution at 4 ºC for 48 h, given laboratory pelleted diet and drinking water for 4 weeks; and group 3 (n = 6), flushed, and stored with EC solution at 4 ºC for 48 h, and given laboratory pelleted diet containing 400 mg of vitamin E/kg for 4 weeks. In the isolated perfused dog kidney model, kidneys were perfused for 2 h at 37.5 ºC, and the glomerular filtration rate (GFR), urinary flow rate (UFR), fractional reabsorbtion of sodium (FRNa+), perfusate flow rate (PFR), renal perfusion pressure (RPP), and released lactic dehydrogenase (LDH) in urine were measured. Our results showed that the functional parameters are very poor after prolonged cold ischemia. Levels of tubular injury marker were significantly higher with a longer ischemic period. In the group given vitamin E, renal functional parameters significantly increased and tubular injury marker in urine significantly decreased. These results indicated that vitamin E supplementation reduced ischemia reperfusion injury in the isolated perfused dog kidney model.

Key Words: Kidney transplantation, cold storage, organ preservation

Introduction

Organ preservation is a key factor in successful kidney transplantation (1). Kidney damage occurs primarily during cold ischemic time (2). The majority of renal transplant centers used cold-stored kidneys with EC solution until 1987 (1). In 1987, Belzer developed University of Wisconsin (UW) solution primarily to extend the preservation times of extrarenal organs such as the liver and pancreas (3). A recent study demonstrated that kidneys preserved by cold storage solutions were not very efficient, and delayed graft function (DGF) was higher than 20% (4). The acute response to perfusion injury involves vascular congestion, tubule injury, and obstruction (5,6). It is defined as a poor graft function with a requirement for dialysis in the immediate post-transplant period (7).

Free oxygen radicals are considered responsible for perfusion injuries (8). Antioxidants may play an important role in preventing free radical damage (9). Vitamin E is a dietary antioxidant in biological systems because of its association with the cell membrane and it prevents lipid peroxidation (10). This study was carried out to investigate the efficacy of vitamin E in kidney preservation.

Materials and Methods

Experimental model. This experimental design was approved by the Ethics Committee of Osmangazi University for the care and use of laboratory animals. Male dogs weighing 18-22 kg were used for the experiments. Intravenous heparin sodium (250 IU) was given before exposure and removal of the kidneys, respectively. General anesthesia was induced with intramuscular ketamine at a dose of 10 mg/kg. Both kidneys were exposed through a midline abdominal incision and were

carefully freed from perirenal tissue and fat. Bilateral nephrectomy was performed with a 2 min warm ischemic interval after which the kidneys were weighed.

Study groups. Dogs were randomized into 3 groups, each containing 6 kidney:

Group 1 (Control group): Animals were given laboratory pelleted diet and drinking water for 4 weeks. Kidneys were immediately reperfused with EC solution after nephrectomy.

Group 2 (Control group): Animals were given laboratory pelleted diet and drinking water for 4 weeks. Kidneys were flushed, and stored with EC solution at 4 ºC for 48 h after nephrectomy.

Group 3: Animals were given laboratory pelleted diet containing 400 mg vitamin E/kg for 4 weeks. Kidneys were flushed, and stored with EC solution at 4 ºC for 48 h after nephrectomy.

Kidney preservation. After the ureter and artery had been cannulated, a kidney was immediately placed in 200 ml of precooled perfusion solution in order to preserve it for 48 h in a sterile basin surrounded by ice in the second and third groups. The cannula in the renal artery was flushed with approximately 250 ml of EC solution at 4 ºC at a pressure of 75 mmHg. A standard EC solution was used for cold storage solutions. EC solution was manufactured by Pharmacie Central des Hôpitaux (Paris, France) and supplied in 1-l bottles. The composition of the solution used was as follows: (mM) 10 Na^+, 115 K^+, 15 Cl^-, 10 HCO_3^-, 50 HPO_4^{2-}, 195 glucose, the resulting pH was 7.0, osmolality was 355 mOsm.

Kidney perfusion. Kidneys were perfused for 2 h. PFR was adjusted to maintain the mean arterial pressure at 75 mmHg. The perfusate temperature during reperfusion was kept at 37.5 ºC and continuously gassed with 5-95% CO_2 that was freshly prepared. Kreps solution adjusted at pH 7.40 contained the following (in mM/l): 140 Na^+, 25 HCO_3^-, 6 K^+, 123 Cl^-, Mg^{2+} 1.2, Ca^{2+} 2.2, D-glucose 11 and 5.0 bm 100 ml of bovine serum albumin (fraction V, Sigma, St. Louis, MO, USA), creatinine 10 mg/l (Sigma, St. Louis, MO, USA) and 5 ml of a 22 l amino acid mixture from a commercial solution (Clintec Nutrition Clinique, Serves, France). Perfusion was provided by a peristaltic pump (Betriebsart, DB; Schutzart: IP21, Germany). RPP was measured by a pressure transducer (Statham P23 Dc, Grass, Quincy, MA, USA). The outflow from the pump passed through a hollow fiber type oxygenator (0600 ECM, Sci-med, Minneapolis, MN, USA). Urine volume was measured at T120 after reperfusion. PFR was monitored, and perfused and urine samples were collected at the same time for biochemical study: creatinine (mg/dl), sodium (mEq/l), release of lactic dehydrogenase (U/l) in urine, and sodium (mEq/l), and creatinine (mg/dl) in perfusate were measured. At the end of reperfusion, the kidneys were weighed again.

Determination of GFR, UFR, $FRNa^+$. Urine samples were collected at T120

- GFR (ml/min) = Urine creatinine (mg/ml) x urine volume (ml/min) / perfusate creatinine (mg/ml)

- UFR (ml/min/g) = 1 min x 1g x urine output (ml) / 60 min x kidney weight

- $FRNa^+$ (%) = Total reabsorbed sodium / Excreted sodium x 100

Total reabsorbed sodium = Filtrated sodium-excreted sodium

Filtrated sodium = GFR x Perfusate sodium

Excreted sodium = Urine sodium x Urine volume

- PFR (ml/min/g) = 1 min x 1g x Perfused output (ml) / 60 min x Kidney weight

Statistical analysis. Data were presented as means ± SE. Analysis of variance (ANOVA) and Tukey tests were used for comparison. Significant results were determined as $P < 0.05$.

Results

The effects of vitamin E on tubular function and glomerular filtration are shown in the Table. The differences between groups 1 and 2 in GFR, UFR and $FRNa^+$ rates were statistically significant ($P<0.001$). GFR, UFR and $FRNa^+$ decreased significantly in group 2. There were significant differences between groups 2 and 3 in GFR, URF and $FRNa^+$ rates ($P<0.001$). GFR, UFR, $FRNa^+$ rates significantly increased in group 3. There were no significant differences between groups 1 and 2 or 2 and 3 in PFR. However, the differences between groups 2 and group 3 RPP levels were significant ($P<0.001$). Vitamin E administration significantly decreased RPP ($P<0.001$). The differences between groups 1 and 2 in % LDH in urine were significant ($P<0.001$). % LDH in urine significantly increased in group 2. There were significant differences between groups 2 and 3 in urine LDH

Table. Effects of vitamin E on glomerular filtration, tubular function, renal vascular function, and tubular injury marker.

	Group 1 (n = 6)	Group 2 (n = 6)	Group 3 (n = 6)
GFR (ml/min)	45.52 ± 5.22	*1.28 ± 0.30	*5.01 ± 1.01
UFR (ml/min/g)	17.83 ± 2.09	*1.47 ± 0.30	*18.17 ± 0.60
$FRNa^+$ (%)	74.32 ± 4.64	*5.82 ± 0.74	*36.67 ± 3.87
PFR (ml/min/g)	1.00 ± 0.10	1.23 ± 0.14	1.15 ± 0.15
RPP (mmHg)	194.00 ± 21.98	146.67 ± 7.56	*55.00 ± 7.31
Urine LDH (U/l)	805.30 ± 158.56	*21779.5 ± 2633.54	*5352 ± 947.91

Values are presented as means ± SE (n = 6/group)

GFR $F_{(2,15)}$= 63.76, * P<0.001; Tamhane post hoc test
UFR $F_{(2,15)}$= 56.76, * P<0.001; Tukey HSD post hoc test
FRNa+ $F_{(2,15)}$= 95.28, * P<0.001; Tukey HSD post hoc test
PFR $F_{(2,15)}$= 0.74, P>0.05; Tukey HSD post hoc test
RPP $F_{(2,15)}$= 25.23, * P<0.001; Tukey HSD post hoc test
Urine LDH $F_{(2,15)}$= 46.47, * P<0.001; Tamhane post hoc test

(P<0.001). When compared to group 2, % LDH in urine significantly decreased in group 3 (Table).

Discussion

Free oxygen radicals are considered responsible for perfusion injuries (8). The acute response to perfusion injury involves attenuation of renal blood flow and GFR, as well as reduced tubular function (11). DGF remains an important complication, which affects about 30-50% of renal transplantations (12). Hauet et al. showed that kidneys preserved in EC for 48 h are not very efficient, and an overall reduction below 20% DGF is not possible with EC cold storage preservation (4). In this study, it was demonstrated that, when compared to the control group, GFR, UFR and $FRNa^+$ rates decreased significantly after the kidneys were flushed with and stored in EC solution at 4 ºC for 48 h. Similarly, cold flushing for 48 h with EC solution decreased RPP. Urinary enzyme excretion, LDH, which is related to tubular cell injury, was significantly increased after the kidneys were flushed with and stored in EC solution at 4 ºC for 48 h. These results demonstrate that glomerular filtration and tubular and vascular functions decreased, and tubular injury increased after prolonged cold storage preservation with EC.

Previous studies showed that the functional damage of glomeruli and microvascular vessels such as renal tubules was related to eicosanoids, which were metabolites of arachidonic acid (AA) synthesized in the kidney cells (13). Phospholipase A_2 (PLA_2) is a rate-limiting enzyme of the AA cascade (14), and PLA_2 activation depends on free radicals or oxidative stress such as lipid peroxidation (15). Vitamin E in blood and tissues acts as an antioxidant that prevents the chain reactions of lipid peroxidation, removes the free radicals and then completes the chain reaction, thereby preventing the lipid peroxidation of polyunsaturated fatty acids in cell membranes (16). Recently, vitamin E supplementation significantly increased GFR, UFR and $FRNa^+$ rates. Cold flushing with EC solution for 48 h decreased RPP and vitamin E administration significantly increased RPP. Urinary enzyme excretion, LDH, which was related to tubular cell injury, was significantly decreased with vitamin E administration.

In conclusion, this study shows that vitamin E administration decreased glomerular, tubular and vascular damage due to ischemic reperfusion injury and that preconditioning the donor with high-dose vitamin E improved the quality of the kidney after reperfusion.

Corresponding author:
Adnan ŞAHİN
Department of General Surgery,
Faculty of Medicine,
Osmangazi University
26480, Eskişehir - Turkey
e-mail: asahin@ogu.edu.tr

References

1. Wahlberg J, Jacobsson J, Tufveson G. Relevance of additive components of University of Wisconsin cold storage solution. An experimental study in the rat. Transplantation 48: 400-3, 1989.
2. Ploeg RJ, Vreugdenhil P, Goossens D et al. Effect of pharmacologic agents on the function of the hypothermically preserved dog kidney during normothermic reperfusion. Surgery 103: 676-83, 1988.
3. Kalayoglu M, Sollinger HW, Stratta RJ et al. Extended preservation of the liver for clinical transplantation. Lancet 19: 617-9, 1988.
4. Hauet T, Mothes D, Goujon JM et al. Evaluation of injury preservation in pig kidney cold storage by proton nuclear magnetic resonance spectroscopy of urine. J Urol 157: 1155-60, 1997.
5. Rabb H, O'Meara YM, Maderna P et al. Leukocytes, cell adhesion molecules and ischemic acute renal failure. Kidney Int 51: 1463-8, 1997.
6. Thadhani R, Pascual M, Bonventre JV. Acute renal failure. N Engl J Med 334: 1448-60, 1996.
7. McKay DB, Milford EL, Tolkoff-Rubin N. Clinical Aspects of Renal Transplantation. (Ed. B. Branner) The Kidney. Saunders Comp. Philadelphia 2000, pp: 2542-605.
8. Baker GL, Corry RJ, Autor AP. Oxygen free radical induced damage in kidneys subjected to warm ischemia and reperfusion. Protective effect of superoxide dismutase. Ann Surg 202: 628-41, 1985.
9. Wang C, Salahudeen AK. Lipid peroxidation accompanies cyclosporine nephrotoxicity: effects of vitamin E. Kidney Int 47: 927-34, 1995.
10. Burton GW, Traber MG. Vitamin E: antioxidant activity, biokinetics, and bioavailability. Annu Rev Nutr 10: 357-82, 1990.
11. Conger JD, Weil JV. Abnormal vascular function following ischemia-reperfusion injury. J Investig Med 43: 431-42, 1995.
12. Kjellstrand CM, Casali RE, Simmons RL et al. Etiology and prognosis in acute post-transplant renal failure. Am J Med 6: 190-9, 1976.
13. Choi JH, Yu BP. Dietary restriction as a modulator of age-related changes in rat kidney prostaglandin production. J Nutr Health Aging 2: 167-71, 1998.
14. Purkerson ML, Hoffsten PE, Klahr S. Pathogenesis of the glomerulopathy associated with renal infection in rats. Kidney Int 9: 407-17, 1976.
15. Shivastava KC. Effects of dietary fatty acids prostaglandins and related compounds on the role of platelet thrombosis. Biochem Exp Biol 16: 317-38, 1980.
16. Kwag OG, Kim SO, Choi JH, Rhee IK et al. Vitamin E improves microsomal phospholipase A2 activity and the arachidonic acid cascade in kidney of diabetic rats. J Nutr 131: 1297-301, 2001.