Transpl Int (1995) 8: 293–297

ORIGINAL ARTICLE

B. M. Stubenitsky
M. Ametani
R. Danielewicz
J. H. Southard
F. O. Belzer

Regeneration of ATP in kidney slices after warm ischemia and hypothermic preservation

Received: 24 July 1994
Received after revision: 1 December 1994
Accepted: 2 December 1994

B. M. Stubenitsky[1] (✉) · M. Ametani
R. Danielewicz · J. H. Southard
F. O. Belzer
Department of Surgery,
University of Wisconsin,
600 Highland Avenue Madison,
WI 53792-3236, USA

Present address:
[1] Department of Surgery,
University Hospital Maastricht,
P. O. Box 5800, 6202 AZ Maastricht,
The Netherlands
Fax: + 31 43 875473

Abstract The current shortage of cadaveric kidneys may be alleviated to some degree by increasing our capabilities to use less than ideal donor kidneys, such as those from non-heart-beating donors. These kidneys are often exposed to no flow (ischemia) for varying lengths of time. Full utilization of these kidneys may require better methods of organ preservation that could reverse existing ischemic injury. This may conceivably require that, during preservation, energy stores (ATP) lost during warm ischemia be recharged. This would require continuous perfusion. Using a kidney slice model, we investigated the effects of simulated hypothermic machine perfusion with the UW gluconate perfusate on the capability of rabbit kidneys exposed to warm ischemia to regenerate ATP. After 30 min of warm ischemia, ATP content was low (0.2 μmol/g wet weight) but increased to 0.7–0.9 μmol/g wet weight after 24 h of simulated machine perfusion at 4 °C. After an additional 2 h of rewarming (37 °C in oxygenated Krebs Henseleit buffer), the slice ATP content increased to about 1.0 μmol/g wet weight (similar to kidneys not exposed to warm ischemia) when the antioxidants desferrioxamine and N-2-(mercaptopropionyl) glycine were included in the preservation media. Significantly less ATP was present without the antioxidants. After 60 min of warm ischemia, less ATP was regenerated after 24 h of simulated machine perfusion (about 0.4 μmol/g wet weight) than after 30 min of warm ischemia. However, more ATP was regenerated when antioxidants were included in the perfusate (0.4 vs 0.8 μmol/g wet weight). This study shows that ATP can be regenerated in kidneys exposed to warm ischemia by continuous perfusion in the UW gluconate solution. Furthermore, oxygen free radicals appear to cause suppression of ATP regeneration since an iron and a hydroxyl radical scavenger improved ATP formation. The ATP content of kidneys exposed to 60 min of WI was less than after 30 min of warm ischemia, suggesting that better methods of preservation may be needed to improve our capability to utilize kidneys damaged by extensive warm ischemia (> 60 min). This may require the development of new methods and/or perfusates for kidney preservation.

Key words Kidney slices, warm ischemia, ATP · ATP, kidney slices, warm ischemia · Ischemia, kidney slices, ATP

Introduction

The shortage of kidneys for transplantation is a mounting problem due to the increasing number of potential recipients without a concomitant number of cadaveric donors. One answer to this problem is the use of less than ideal kidneys, such as those from non-heart-beating cadavers. Kidneys from these donors will have been exposed to varying lengths of warm ischemia, followed by cold preservation.

The best method to preserve warm ischemically damaged kidneys may be continuous hypothermic machine perfusion. This method removes end products of ischemic metabolism and allows the kidney to maintain

or regenerate critically important metabolites lost during warm ischemia, such as adenine nucleotides, glutathione, etc. The perfused kidney may be able to repair existing damage upon reperfusion, such as re-acylation of fatty acid components of phospholipids that are thought to break down during warm ischemia [18]. However, the repair of damage will require a source of energy, namely, ATP. Therefore, a critical factor in utilizing kidneys from non-heart-beating cadavers may be stimulating ATP regeneration during hypothermic machine perfusion.

The aim of this study was to evaluate the ability of warm ischemic kidneys to regenerate ATP during hypothermic machine perfusion with University of Wisconsin gluconate perfusate (UW-gluconate), as well as upon normothermic reperfusion. It has been suggested that oxygen free radicals (OFR) and, in particular, hydroxyl radicals [1, 7, 12], cause much of the injury associated with ischemia and reperfusion [4, 5, 13, 17, 21, 22]. The inability to regenerate ATP after warm ischemia may, therefore, be due to damage inflicted by OFR release.

We studied the effects of adding to the perfusion solution the free radical scavengers desferrioxamine (Des), an iron chelator, and N-2-(mercaptopropionyl) glycine (MPG), a hydroxyl radical scavenger, on the regeneration of ATP. Rabbit kidney slices were prepared from warm ischemically damaged kidneys and stored in UW-gluconate (UW-gluconate plus and minus antioxidants) with continuous shaking to simulate machine perfusion. ATP regeneration was measured as an indication of the metabolic competency of the tissue. The capability of warm ischemically damaged kidneys to regenerate ATP during hypothermic perfusion may be a first step in repairing ischemic damage. In order to fully utilize kidneys from non-heart-beating cadavers, a reasonable goal may be to stimulate ATP production during preservation.

Materials and methods

Kidney preparation

New Zealand white rabbits weighing 2–2.5 kg, with free access to food and water, were used in these experiments. The principles of laboratory animal care were followed according to NIH publication no. 85-23, revision 1985. The animals were anesthetized using nembutal, followed by succinyl choline, and the kidneys were removed by a midline abdominal incision. The rabbits were heparinized (1000 IU, i. v.) and the kidneys rapidly removed and flushed out (15 ml from a height of 50 cm) with either cold preservative (4 °C, UW-gluconate) or 37 °C saline. The kidneys flushed out cold were immediately used for slice preparation. The kidneys flushed out at normothermia were placed in a 37 °C water bath for either 30 or 60 min, after which they were used for slice preparation.

Slice preparation

Thin (< 0.5 mm) renal cortical slices of approximately 0.04 g each were prepared using a Stadie Riggs tissue slicer. After preparation, the slices were rinsed in saline and placed in UW-gluconate solution at 4 °C. The composition of this solution is identical to that described by McAnulty et al. [19] for continuous machine perfusion of kidneys. Five slices were placed in 5 ml of preservative with continuous shaking (60 oscillations per minute) in open 25-ml erylenmeyer flasks. The oxygen content of this solution during shaking measured 150 torr. Each flask was gently shaken for 24 h to simulate continuous machine perfusion.

Three different solutions were used: UW-gluconate (group 1); UW-gluconate with Des (1 mM, group 2); and UW-gluconate with Des (1 mM) and MPG (1 mM, group 3).

Rewarming

After preservation, slices were either frozen in a dry-ice acetone bath for later analysis of ATP or rinsed in saline and placed in flasks containing Krebs-Henseleit buffer (KHB) at 37 °C. The composition of KHB used was: 118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgCl_2$, 11.1 mM glucose, 10 mM $NaHCO_3$, and 20 mM HEPES; pH 7.4. The slices were incubated under an atmosphere of O_2 : CO_2 (95 % : 5 %) for 120 min with continuous shaking (simulated reperfusion) and were thereafter used for ATP measurement.

ATP analysis

ATP was separated from acid-precipitated renal cortical tissue samples by high performance liquid chromatography using a 15-cm Supelcosil ion exchange column (Supelco SA) with 5-μm particle size and a 3-cm guard column [23]. The concentrations of ATP were determined by peak area integration and compared to the peak area of a known standard of ATP. No significant conclusions could be drawn from analysis of ATP-related compounds (i. e., ADP, AMP); therefore, this data was not included to allow simplification of presentation.

Statistical analysis

Results are presented as means with standard error of the mean (SEM) for at least four kidneys in each group. Comparison of means between groups was calculated with the one-way analysis of variance (ANOVA) test with a P level below 0.05 being considered significant. Further analysis to determine differences within the group were done with the Tukey-Kramer Multiple Comparisons Test ($P < 0.05$ considered significant).

Results

The effects of 0 min (control), 30 min, and 60 min of warm ischemia WI on the ATP content of kidney slices after 24 h of simulated machine perfusion (sMP) and 2-h rewarming are shown in Fig. 1. In control slices (Fig. 1 a), the ATP content was about 1.3 μmol/g wet weight, and after 24 h at 4 °C (sMP) in plain UW there was a decline in the ATP content to 0.9 μmol/g wet

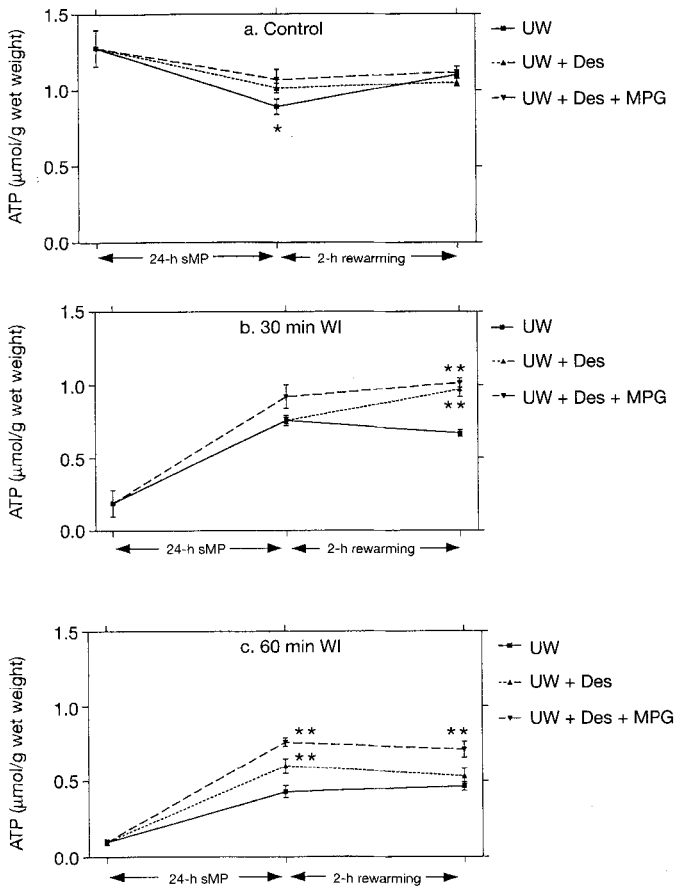

Fig. 1 a–c ATP content of rabbit kidney slices measured under the following conditions: no preservation (control), after 24-h cold preservation (sMP), and after 2-h normothermic incubation. The preservation solutions are as shown. Kidneys were exposed to warm ischemic intervals of: **a** 0 min, **b** 30 min, and **c** 60 min prior to slice preparation. Values are presented as means ± SEM for at least four kidneys per group. * $P < 0.05$ versus 0 time preservation; ** $P < 0.05$ versus UW at the same time point

weight, which was significantly less than the 0 time ATP content ($P < 0.05$). There was less of a decline in ATP content after 24 h of sMP when the preservation solution contained antioxidants (Des or Des + MPG), and the differences were not significantly different from the 0 time ATP content. After 2 h of rewarming, the ATP content was similar to the content after sMP in all three groups.

After 30 min of warm ischemia (Fig. 1 b), the ATP content of the kidney had declined to about 0.2 μmol/g wet weight. After 24 h of hypothermic preservation (sMP), the ATP content increased to about 0.8–0.9 μmol/g wet weight in slices preserved in UW-gluconate or UW with the antioxidants. This concentration of ATP was similar to that seen in control slices (± antioxidants) after 24 h of sMP ($P > 0.05$). When rewarmed, the ATP content decreased slightly in the slices that were preserved in the UW-gluconate. However, in slices preserved with the antioxidants, ATP increased after rewarming and was significantly greater ($P < 0.05$) than in slices preserved without the antioxidants.

After 60 min of warm ischemia (Fig. 1 c), the ATP content of the kidney had declined to about 0.1 μmol/g wet weight. There was some ATP regeneration during the 24 h of hypothermic preservation (sMP), after which the ATP content was about 0.4 μmol/g wet weight. When preserved with the antioxidants, the ATP content of the slices after 24 h was greater (0.6–0.8 μmol/g wet weight) than without antioxidants and the differences were significant ($P < 0.05$). Following rewarming, the ATP content remained nearly identical to the ATP content after hypothermic preservation ($P > 0.05$). The ATP content in the slices preserved in UW plus Des + MPG and rewarmed, however, was significantly greater than in the other two groups after rewarming.

Discussion

This study shows that under hypothermic conditions that simulate machine perfusion of the kidney, slices from kidneys exposed to warm ischemia can effectively regenerate ATP. The amount of ATP regenerated after 30 min for warm ischemia and hypothermic preservation (sMP) was similar to the amount of ATP in slices from kidneys not exposed to warm ischemia (controls). Rewarming of the slices for 2 h after 24-h preservation resulted in ATP levels similar to slices from control or 30-min warm ischemia kidneys. Therefore, 30 min of warm ischemia does not appear to cause significant injury to the energy-synthesizing capabilities of the rabbit kidney. This degree of warm ischemia followed by machine perfusion is well tolerated by human [14] and dog [16] kidneys, as shown in a transplant model. After 60 min of warm ischemia, however, less ATP is regenerated than after 30 min of warm ischemia. It has been shown that dog kidneys exposed to 60 min of warm ischemia and 24-h perfusion do not survive in a transplant model [16]. The inability of the kidney to regenerate ATP may be an indicator of loss of viability due to the combination of warm ischemia and hypothermic preservation.

There is increasing evidence that oxygen derived free radicals are generated upon reperfusion of kidneys exposed to warm ischemia [2, 4, 5, 9, 13, 17, 20–22], and the origin of these may be iron-mediated [1, 7, 12], with injury being caused by hydroxyl radicals [12]. Although the generation of OFR would be slowed by hypothermia [6], the amount of exposure of the kidney to the cold (24 h) could be sufficient to produce a significant concentration of these potentially cytotoxic radicals. Thus, in this study, we chose to study two antioxidants.

One, desferrioxamine mesylate, reduces the toxic effects of iron and inhibits hydroxyl radical formation via the Haber-Weiss reaction, as well as via lipid peroxidation pathways [1, 8, 11]. Green et al. [10] have shown that lipid peroxidation was suppressed in ischemically damaged rabbit kidneys treated with Des. The other antioxidant, N-2-(mercaptopropionyl) glycine, has been shown to effectively scavenge hydroxyl radicals directly and has been used in reversing myocardial reperfusion damage [15] and preventing mitochondrial injury following ischemia and reperfusion in hepatocytes [25, 27]. MPG readily crosses the cell membrane and can accumulate in the mitochondria [26], thus exerting its hydroxyl radical scavenging action within the cell and organelles.

These two antioxidants appear to be benefical to ATP regeneration in hypothermically preserved rabbit kidney slices. In control slices, the ATP content after 24 h of sMP was higher when incubated with the antioxidants than without. In slices from kidneys exposed to warm ischemia for 30 min, the presence of the antioxidants showed higher ATP after cold preservation (not significant) and after rewarming (significant). Even after 60 min of warm ischemia, the presence of the antioxidants in the UW-gluconate solution resulted in greater ATP regeneration. These results suggest that OFR are produced during cold perfusion of kidneys and that they can affect metabolism in an adverse way (i.e., by reducing ATP synthesis).

In conclusion, there is a shortage of organs for transplantation. The utilization of kidneys from non-heart-beating cadavers, or less than ideal kidneys, could help to alleviate this shortage. We suggest here that the most effective means of preserving these kidneys may require machine perfusion [3, 24]. However, the current method of machine perfusion may not be adequate, and newly developed perfusion fluids may be required. Machine perfusion of damaged kidneys may help "recharge" these organs by enhancing ATP production during cold preservation. Although hypothermia slows down metabolism, it is clear that ATP synthesis continues and may be enhanced by the presence of OFR scavengers in the perfusate. Stimulating ATP synthesis in the ischemically damaged kidney may be necessary to provide energy for the repair of damage caused by warm ischemia and to maintain the structural integrity of the cell. In the future, other additives to the perfusion fluid may be necessary to further increase the tolerance of the warm ischemically injured kidney to hypothermic perfusion.

Acknowledgements This work was supported by NIH grant DK 18624. The first author would like to thank Dr. M.H. Booster for his consideration and influence that led the first author to go to the United States to complete this research under the direction of Dr. James Southard.

References

1. Aust SD, Morehouse LA, Thomas CE (1985) Role of metals in oxygen radical reactions. Free Radic Biol Med 1: 3–25
2. Baker GL, Corry RJ, Autor AP (1985) Oxygen free radical induced damage in kidneys subjected to warm ischemia and reperfusion. Protective effect of superoxide dismutase. Ann Surg 202: 628–641
3. Barber WH, Laskow DA, Deierhoi MH, Poplawski SC, Diethelm AG (1991) Comparison of simple hypothermic storage, pulsatile perfusion with Belzer's gluconate-albumin solution, and pulsatile perfusion with UW solution for renal allograft preservation. Transplant Proc 23: 2394–2395
4. Baron P, Gomez-Marin O, Casas C, Heil J, Will N, Condie R, Burke B, Najarian JS, Sutherland DE (1991) Renal preservation after warm ischemia using oxygen free radical scavengers to prevent reperfusion injury. J Surg Res 51: 60–65
5. Bosco PJ, Schweizer RT (1988) Use of oxygen radical scavengers on autografted pig kidneys after warm ischemia and 48-hour perfusion preservation. Arch Surg 123: 601–604
6. Cadenas E, Varsavsky AI, Boveris A, Chance B (1981) Oxygen- or organic hydroperoxide-induced chemiluminescence of brain and liver homogenates. Biochem J 198: 645–654
7. Diguiseppi J, Fridovich I (1980) Ethylene from 2-keto-4-thiomethyl butyric acid: the Haber-Weiss reaction. Arch Biochem Biophys 205: 323–329
8. Fuller BJ, Lunec J, Healing G, Green CJ (1987) Reduction of susceptibility to lipid peroxidation by desferoxamine in rabbit kidneys subjected to 24-hours cold ischemia and reperfusion. Transplantation 43: 604–606
9. Green CJ, Healing G, Lunec J, Fuller J, Simpkin S (1986) Evidence of free radical induced damage in rabbit kidneys after simple hypothermic preservation and autotransplantation. Transplantation 41: 161–165
10. Green CJ, Healing G, Simpkin S, Fuller BJ, Lunec J (1986) Reduced susceptibility to lipid peroxidation in cold ischemic rabbit kidneys after addition of desferoxamine, mannitol, or uric acid to the flush solution. Cryobiology 23: 358–365
11. Gutteridge JM, Richmond R, Halliwell B (1979) Inhibition of the iron catalyzed formation of hydroxyl radicals from superoxide and of lipid peroxidation by desferoxamine. Biochem J 184: 469–472
12. Halliwell B (1978) Biochemical mechanisms accounting for the toxic action of oxygen on living organisms: the key role of superoxide dismutase. Cell Biol Int Rep 2: 113–128
13. Hansson R, Gustafsson B, Jonsson O, Lunstam S, Petterson S, Schersten T, Waldenstrom J (1982) Effect of xanthine oxidase inhibition on renal circulation after ischemia. Transplant Proc 14: 51
14. Hoffmann RM, Stieper KW, Johnson RW, Belzer FO (1974) Renal ischemic tolerence. Arch Surg 109: 550–551

15. Horwitz LD, Fennessey PV, Shikes RH, Kong Y (1994) Marked reduction in myocardial infarct size due to prolonged infusion of an antioxidant during reperfusion. Circulation 89: 1792–1801
16. Johnson RWG, Anderson M, Morley AR, Taylor RM, Swinney J (1972) Twenty-four-hour preservation of kidneys injured by prolonged warm ischemia. Transplantation 13: 174–179
17. Lunec J, Fuller B, Green CJ (1983) Evidence of lipid peroxidation damage in rabbit kidneys during warm ischemia. Cryobiology 20: 731
18. Mathys E, Patel Y, Kreisberg J, Stewart JH, Ventkatachalam M (1984) Lipid alteration induced by renal ischemia. Pathogenic factor in membrane damage. Kidney Int 26: 153–161
19. McAnulty JF, Ploeg RJ, Southard JH, Belzer FO (1989) Successful five-day perfusion of the canine kidney. Transplantation 47: 37–41
20. McCord JM (1985) Oxygen derived free radicals in postischemic tissue injury. N Engl J Med 312: 159–163
21. Ouriel K, Smedira NG, Ricotta JJ (1985) Protection of the kidney after temporary ischemia: free radical scavengers. J Vasc Surg 2: 49–53
22. Paller MS, Hoidal JR, Ferris TF (1984) Oxygen free radicals in ischemic acute renal failure in the rat. J Clin Invest 74: 1156–1164
23. Sellevold OFM, Jynge P, Aarstad K (1986) High performance liquid chromatography: a rapid isocratic method for determination of creatine compounds and adenine nucleotides in myocardial tissue. J Mol Cell Cardiol 18: 517–527
24. Southard JH, Belzer FO (1994) Organ preservation. Ann Rev Med (in press)
25. Starke PE, Farber JL (1985) Endogenous defenses against the cytotoxicity of hydrogen peroxide in cultured rat hepatocytes. J Biol Chem. 260: 86–92
26. Williams R, Gasbarrini G, Davis M (1981) Recent advances in 2-MPG treatment of liver diseases. Editrice Compostitori, Bologna, pp 1–383
27. Zimmer G, Beyersdorf F, Fuchs J (1985) Decay of structure and function of heart mitochondria during hypoxia and related stress and its treatment. Mol Physiol 8: 495–513