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Improved renal preservation with PB-3 flush solution during 72 h of cold storage

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Abstract Previously, PB-2 flush solution has been found to be superior to Collins 2 solution (C-2) in extending renal viability in the dog. To further characterize preservation mechanisms, we studied mitochondrial oxidative function during 72 h of cold storage comparing PB-3, UW-1, and C-2 flush storage solutions. Complex 1 dependent mitochondrial oxidative phosphorylation (MOP) was found to be significantly less ($P < 0.001$) at 5 h and 72 h of cold storage ($P < 0.001$) for the C-2 compared to the other flush groups. Complex 2 dependent MOP had parallel results, having significantly less function ($P < 0.002$) at 5 h and 72 h ($P < 0.02$) of cold storage in the C-2 group compared to the other groups. PB-3 and UW-1 solutions were noted to be comparable, suggesting possible equivalent preservation efficacy. Nevertheless the components of PB-3 at the level of MOP contributed significantly to better preservation compared to C-2 solution.

Key words Renal transplantation
Organ preservation · Intracellular flush solution

Introduction

Previously, we have presented a modified intracellular flush solution (PB-2) that was found to be superior to Collins (C-2) solution in extending renal viability during cold storage in the dog [1]. Graft survival is significantly greater in the PB-2 group compared to the C-2 group ($P < 0.01$). Recovery of renal function in the PB-2-treated group is also significantly faster compared to the C-2 group. HPLC studies of canine kidneys have revealed superior regeneration of adenine nucleotides with PB-2 during 5–45 min of reperfusion, while further deterioration was noted in the same time interval in the C-2 group, characteristic of a "reperfusion injury" [1]. In summary this previous study has shown that the mechanisms involved included both diminution of reperfusion injury and maintenance of intracellular high energy metabolites.

PB-3 solution has recently been introduced. It is a mannitol-based intracellular solution as is the PB-2 solution, with the addition of a xanthine oxidase inhibitor (allopurinol) and calcium entry blocker (verapamil) compounds. Composition of different solutions (C-2, UW-1, PB-2, PB-3, and other renal flush solutions) are shown in Table 1 (from reference [1]). Preliminary results in five canine autotransplants after 72 h of simple cold storage using PB-3 flush solution, and contralateral nephrectomy yielded 100% recovery and survival (personal communication).

The purpose of this study was to further characterize the preservation mechanisms involved in PB-3 compared to other well-known solutions. In order to accomplish

Table 1 Composition of different intracellular renal flush solutions (g/l)

	PB-2 flush	Collins-2 flush	Sacks-2 flush	Belzer perfusate	UW-1 flush	PB-3 flush
KH_2PO_4	2.05	2.05	4.16	3.4	3.4	2.05
$K_2HPO_4 \cdot 3H_2O$	9.70	9.70	–	–	–	9.70
KCl	1.12	1.12	–	–	–	1.12
$KHCO_3$	–	–	2.30	–	–	2.30
Mannitol	25.0	–	37.5	–	–	25.0
Glucose	–	25	–	1.5	–	–
$MgSO_4 \cdot 7H_2O$	3.70	7.38	–	8	1.2	–
$MgCl_2$	(2 mEq/ml)	–	(2 mEq/ml)	–	–	(2 mEq/ml)
Adenosine	1.0	–	–	1.3	1.34	1.0
Sodium glutathione	–	–	–	17.5	0.92	–
Albumin	–	–	–	5.3	–	–
$NaHCO_3$	0.84	0.84	1.26	–	–	0.84
Allopurinol	–	–	–	0.113	0.113	0.100
Verapamil	–	–	–	–	–	0.007
K^+-lactobionate	–	–	–	–	39.8	–
Raffinose	–	–	–	–	17.8	–
Hydroxyethyl starch	–	–	–	–	50	–
Osmolality (mOsm/kg)	340	320	430	300	320–330	340
pH	7.25	7.00	7.00	7.10	7.40	7.30–7.40

this we studied mitochondrial oxidative function during simple cold storage, comparing PB-3, UW-1, and C-2 flush storage solutions.

Materials and methods

Sixty kidneys from adult male Sprague-Dawley rats were cold flushed in situ (PB-3, $n = 20$; UW-1, $n = 20$; C-2, $n = 20$) and then cold stored. Mitochondrial oxidative phosphorylation (MOP) rates were monitored during 72 h of cold storage. At specific intervals, mitochondria were isolated from the renal cortex by differential centrifugation as previously described [2]. Respiration state 3 O_2 uptake was measured with a Clark electrode chamber. Mitochondrial concentration was indexed to cytochrome C (a and a3) measured from the oxidized – reduced optical density from a dual wave spectrometer at 540–510 nm, using a standardized extinction coefficient [3]. Complex I (glutamate/malate substrate) and complex II (succinate substrate) functions were assayed separately. Respiratory (acceptor) control index was calculated using the state 3 (excess substrate and ADP)/state 4 ratios.

Results

Complex I dependent MOP were found to be significantly greater in the PB-3 and UW-1 groups during cold storage compared to the C-2 group. MOP at 5 h (baseline) was 141 ± 10 ng O_2/mg per minute for PB-3 and 138 ± 9 for UW-1 vs. 70 ± 3 for C-2 ($P < 0.0001$) and MOP at 72 h was 105 ± 15 for PB-3 and 108 ± 9 for UW-1 vs. 66 ± 9 for C-2 ($P < 0.0001$; Fig. 1). Complex II dependent MOP had

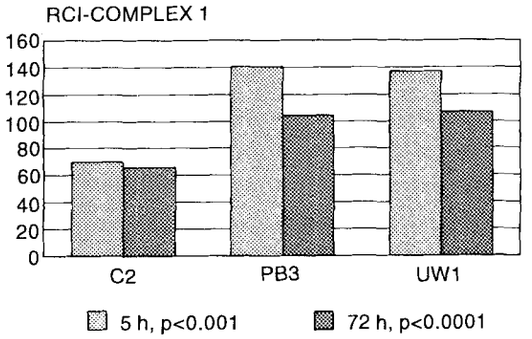

Fig. 1 RCI (respiratory control index) revealed significantly superior mitochondrial renal viability in PB-3 and UW-1 compared to C-2 groups (complex I)

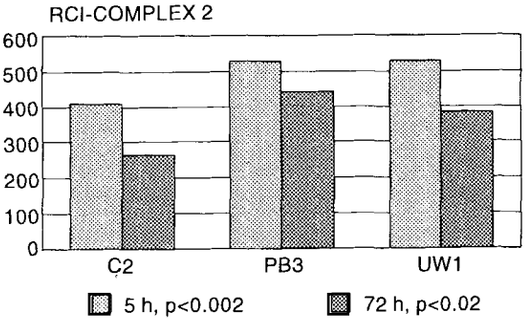

Fig. 2 RCI (respiratory control index) revealed significantly superior mitochondrial renal viability in PB-3 and UW-1 compared to C-2 groups (complex 2)

parallel results with 533 ± 48 for PB-3 and 535 ± 30 for UW-1 vs. 411 ± 10 for C-2 ($P < 0.002$) at 5 h, and 446 ± 82 for PB-3 and 390 ± 74 for UW-1 vs. 266 ± 20 for C-2 at 72 h cold storage ($P < 0.02$; Fig. 2). There were no significant differences between PB-3 and UW-1 solutions.

Discussion

Respiratory or acceptor control ratios or indexes are the rate of respiration of mitochondria in the presence of ample ADP to the rate of respiration in the absence of ADP. These respiratory control indexes (RCI) are normally high (100–500) in viable mitochondria. However, when damaged, the mitochondria lose their ability to phosphorylate ADP and the RCI's fall to 1–100. In general, the higher the RCI, the more functionally intact are the mitochondria. By using isolated mitochondria versus the whole renal unit in preservation studies, nonreperfusion mechanisms may be studied. Preservation additives in PB-3 known to diminish the reperfusion injury are mannitol, verapamil, and allopurinol. Adenosine is the only additive not in C-2 and found in both UW-1 and PB-3. Its presence may contribute significantly ($P < 0.002$) to the observed improved mitochondrial respiration during cold storage (Fig. 3).

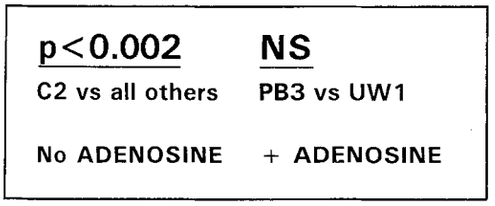

Fig. 3 Adenosine was associated with significant improvement in mitochondrial respiration during cold storage

A possible mechanism for improved mitochondrial respiration with adenosine is preservation of energy charge (ATP/ADP). Another possible mechanism is the slowing of glycolysis via conformational changes in associated enzymes such as phosphofructokinase or other intramembranous proteins used in oxidative phosphorylation, thus slowing metabolism. These mechanisms may be involved in the observed clinical reduction in acute tubular necrosis using UW-1 compared to Euro-Collins solution [4].

In summary, these findings suggested that components of PB-3 significantly contribute to better renal preservation during cold storage compared to C-2 solution at the level of mitochondrial oxidative phosphorylation. These mechanisms are probably completely separate from free radical scavenger and "reperfusion injury" mechanisms. The comparable results seen between PB-3 and UW-1 solution suggested possibly equivalent clinical efficacy. However trials are required to verify this contention.

References

1. Bretan PN, Baldwin N, Martinez A et al (1991) Improved renal transplant preservation using a modified intracellular flush solution (PB-2). Urol Res 19:73–80
2. Chance B (1965) Reaction of oxygen with the respiratory chain in cells and tissues. J Gen Physiol 49:163
3. Mela L, Goodwin CW, Miller LD (1976) In vivo control of mitochondrial enzyme concentrations and activity by oxygen. J Physiol 231:1811–1815
4. Moukarzel M, Benoit G, Bensadoun H et al (1990) Non-randomized comparative study between University of Wisconsin cold storage and Euro-Collins solution in kidney transplantation. Transplant Proc 22:2289–2290