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Improved renal transplant preservation using a modified intracellular flush solution (PB-2)

Characterization of mechanisms by renal clearance, high performance liquid chromatography, phosphorus-31 magnetic resorance spectroscopy, and electron microscopy studies

P. N. Bretan Jr.[1,4], N. Baldwin[3], A. Martinez[1], N. Stowe[1], A. Scarpa[4], K. Easley[2], E. Erturk[1], C. Jackson[1], J. Pestana[1], and A. C. Novick[1]

Departments of [1]Urology, and [2]Biostatistics, and the [3]Magnetic Resonance Research Center, the Cleveland Clinic Foundation, and [4]Department of Biophysics and Physiology, Case Western Reserve University, Cleveland, Ohio, USA

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Summary. A number of new intracellular renal flush solutions have been found to be more efficacious than Collins-2 (C-2) solution in extending organ viability during simple cold storage. However, the mechanism of action of these solutions remains poorly understood. To delineate better underlying intracellular mechanisms, we studied a modified, simple, hypothermic, intracellular (340 mOsm/kg) flush solution (PB-2). The development of PB-2 solution is based on the ability of some of its individual components to minimize ischemic adenine nucleotide (AN) catabolism and endothelial post "reperfusion injury." Preliminary results in 10 canine autorenal transplants show a significant ($P < 0.02$) improvement in renal recovery and viability (recipient posttransplant inulin clearance and survival) after 50 h of cold storage compared with 10 canine kidneys similarly preserved using conventional C-2 flush solution. High performance liquid chromotography (HPLC) studies show a significant ($P < 0.01$) loss of AN using C-2, while PB-2 was associated with regeneration of AN within 45 min of reperfusion. Magnetic resonance spectroscopy using phosphorus 31 (^{31}P-MRS) showed more high energy phosphorus metabolites (phosphomonoester and nicotinamide-adenine-dinucleotide phosphate: $P < 0.001$) at 50 h cold storage using PB-2 compared with C-2. Electron micrographs (EM) revealed normal microcapillary morphology for the PB-2 group; however, moderate vascular red and white blood cell clumping was observed in the C-2 group. Characterization of the basic preservation mechanisms by HPLC, ^{31}P-MRS, and EM studies indicates that PB-2 solution enhances renal preservation by diminution of both reperfusion injury and the loss of intracellular high energy metabolites that are necessary for viability.

Key words: Renal transplantation – Organ preservation – Magnetic resonance spectroscopy (^{31}P-MRS) – Intracellular flush solutions

Organ preservation has become a more important issue in the cyclosporin (CYA) era of renal transplantation. For example, with the use of CYA, a synergistic nephrotoxic effect is noted when a significant ischemic insult is sustained by a donor kidney prior to transplantation [16, 30, 31]. In addition, there is increasing evidence that moderate ischemic injury may predispose renal allografts to severe rejection and diminished survival [4, 17, 23, 31]. For non renal organs, such as the pancreas, liver, and heart, preservation is even more important. An increase in the safe preservation window of these organs would extend the geographical limits for procurement and would facilitate sharing between transplant centers, both regionally and nationally.

Recent research methods, such as magnetic resonance spectroscopy using phosphorus 31 (^{31}P-MRS) [5–7], electron microscopy (EM) [5, 28] and high performance liquid chromatography (HPLC) [26, 40] have elucidated important cellular mechanisms integral to successful organ preservation (Figs. 1, 2) [4]. These studies have illustrated that organ preservation maneuvers are important, not only during cold storage but also during the immediate (1–3 h) posttransplant reperfusion period [20, 25]. It is postulated that a preventable and reversible reperfusion injury occurs at this time. These interactions (Figs. 1, 2) occuring at the intracellular metabolic level must be considered in the development of new and improved flushing solutions for renal preservation.

We propose a modified intracellular cold flush solution (PB-2) designed to minimize adenine nucleotide (AN) catabolism and postpreservation renal "reperfusion injury." The individual components of PB-2 are all nontoxic and have been found to be stable for storage indefinitely (> 14 months). PB-2 composition is compared with similar [i.e., Sack's, Collins-2 (C-2), and UW-1] and currently used intracellular cold flush solutions [3, 11–14, 18, 19, 35] (Table 1). The purpose of this study was twofold: (1) to compare the renal preservative efficacy of PB-2 flush with that of C-2 in an animal model; (2) to define mechanisms of renal preservation using ^{31}P-MRS, EM, HPLC, and inulin clearance studies.

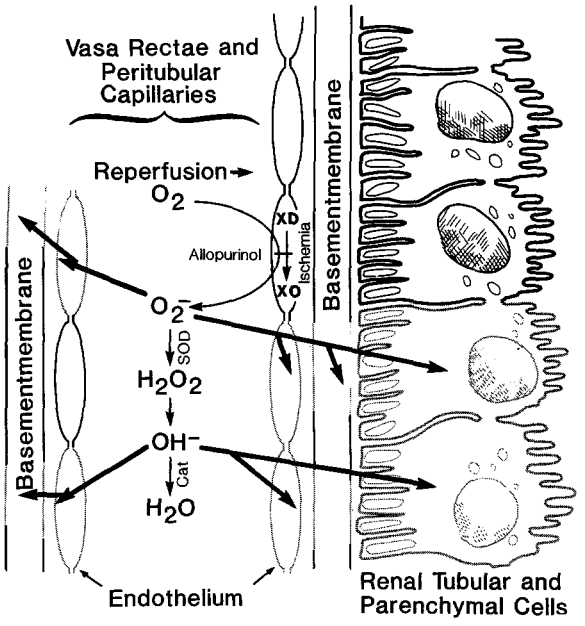

Fig. 1. Mechanism of renal reperfusion injury after transplant revascularization. From [4], with permission. See discussion for details

Materials and methods

Surgical procedure

Twenty female laboratory dogs (18–22 kg) were used. A hydrating solution of 5.0% dextrose in 0.45% saline at 75 cc/h was given intravenously throughout all procedures. One kidney was exposed by a midline abdominal incision, then removed and flushed with PB-2 at 4°C (adenosine-$MgCl_2$, mannitol intracellular cold flush, see Table 1) or C-2 solution.

The animals were divided into two groups, and the kidneys were flushed with specific cold storage solutions as follows: group I ($N=10$) controls - kidneys flushed and cold stored with C-2 solution; group II ($N=10$) kidneys flushed and cold stored with PB-2 solution.

The animals recovered and were fed ad libitum. The ex vivo kidneys in both groups were subjected to renal viability analysis by ^{31}P-MRS, EM, and HPLC studies after 50 h of simple hypothermic storage. Following this initial viability assessment, the animals were reanesthetized, and the midline abdominal incision was reopened.

After contralateral transperitoneal nephrectomy, the preserved kidney was autotransplanted ino the right iliac fossa using the iliac artery and vein. An extra vesicle ureteroneocystostomy was also constructed. Serial HPLC and EM exams from renal tissue wedge biopsies were performed during the immediate reperfusion period in both groups.

Survival and renal function studies

After transplantation the abdominal incision was closed, and the animals were followed for up to 22 days posttransplantation with serial renal function studies, such as the serum creatinine concentration (measured daily photometrically using an AMES instrument) and the glomerular filtration rate (GFR; determined by clearance calculations of inulin) [15]. GFR measurements were taken as a baseline prior to surgery and after transplantation every 2–4 days, if the animals survived and were stable. Animal survival was recorded as the number of days after surgery. At a maximum of 22 days posttransplantation, all animals were sacrificed, and gross and histopathologic examinations of the transplanted kidneys were performed.

High performance liquid chromatography studies

During (a) normal insitu, (b) after 50 h cold storage, (c) after 5 min, and (d) after 45 min of revascularization of the transplanted kidney, renal wedge biopsies were taken from all transplanted kidneys to measure tissue AN, xanthine, and hypoxanthine using HPLC. The latter two metabolites are participants in the formation of toxic free radicals.

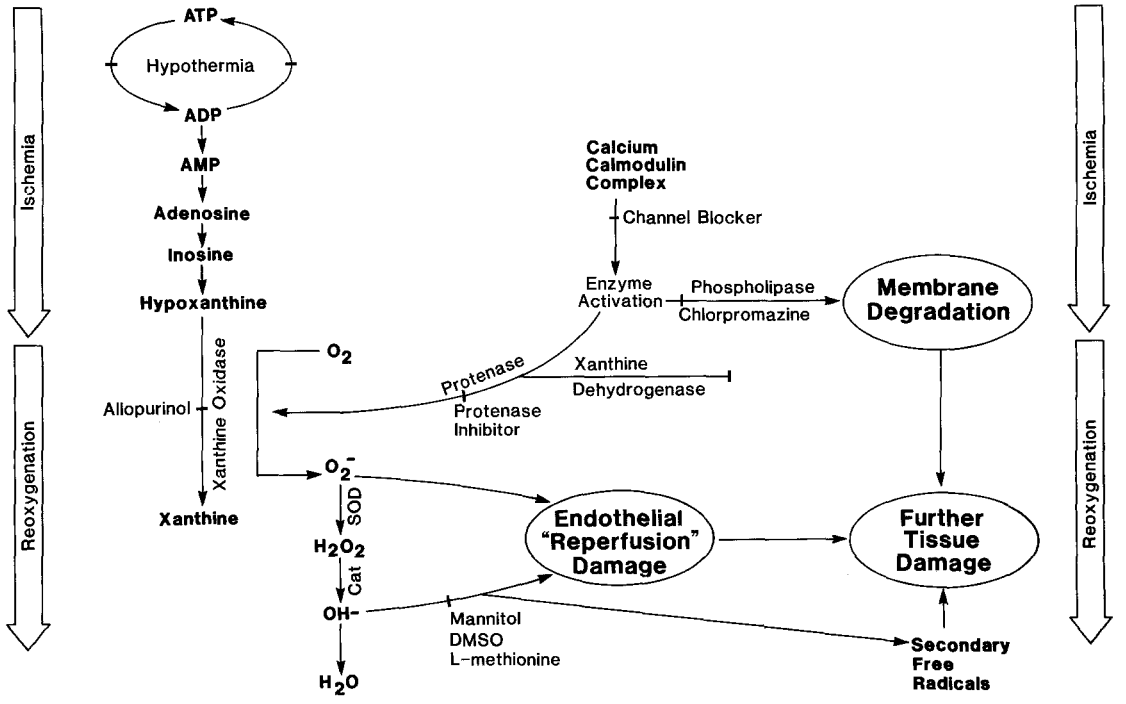

Fig. 2. Proposed metabolic pathways involved in renal transplant preservation. From [4] with permission. See discussion for details

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	–	–	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

Freeze-clamped (−200 °C) renal specimens were ground to a fine powder with a cooled (−100 °C) mortar and pestle. AN were extracted from (25–35 mg) portions, using 1 ml of ice cold 2 *N* perchloric acid with 1 m*M* ethylenediaminetetraacetic acid (EDTA), then neutralized with 1 ml of 2 *N* $KHCO_3$ to pH 6.90 ± 0.10 and centrifuged at 8,500 rpm for 4 min. Aliquots of 15 µl of resulting supernatant was analyzed by HPLC using a Waters system equipped with a 5 µm C18 reversed phase column. An isocratic system was applied using 0.3 *M* NH_4PO_4 as the mobile phase, titrated with NaOH to pH 6.40. The mobile phase was pumped at 2 ml/min, and all separations occurred within 9 min. Peaks heights were measured at 215 nm and quantitated against an external standard (ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; HYPO, hypoxanthine; XAN, xanthine) solutions of known concentrations. Total adenine nucleotides (TAN = sum of ATP, ADP, and AMP), and degradation products sum (DP = sum of HYPO and XAN) were calculated from measured concentrations. The frozen specimens were weighed using a hanging balance scale (Mettler Instrument Corp.) and from this the dry tissue weights calculated (0.60 of wet weight). Concentrations were standardized to micromoles/gram dry weight tissue [26, 40].

Magnetic resonance spectroscopy

^{31}P-MRS studies of intact canine kidneys were acquired after 50 h hypothermic storage, just prior to autotransplantation. Intracellular phosphorus metabolites were directly monitored by ^{31}P-MRS in whole canine kidneys using a 2 cm MRS coil placed just external to the cold storage capsule containing the preserved kidney. The midportion of the kidney was centered directly over the coil, enabling monitoring of approximately 100 ml of renal cortex and medulla. Spectra were obtained at 81 MHz on a General Electric CSI-2 spectrometer using a 4.7-T, 40-cm (horizontal bore), superconducting magnet. The H_2O signal was used to shim the magnet before acquiring the ^{31}P spectra.

Signal-to-noise was optimized using a tip angle of 80 µs. Fourier transforms of 256 acquisitions with a 3-s recycle time were used for measurements. A total of 1,024 data points was collected during 64 ms over a spectral width of 4,000 Hz, which were Fourier transformed (usually with no line boradening) to 4096 points in the frequency domain. Spectra typically included peaks from intracellular nicotinamide-adenine-dinucleotide phosphate (NADP), phosphomonoester (PME), and inorganic phosphate (P_i).

The P_i peak observed in these spectra was composed of two components: one arising from intracellular P_i and the other from P_i in the buffer. The latter could be easily identified from an additional spectrum taken under the same conditions but with the storage container set slightly to the side, so the kidney was away from the center of the surface coil. This peak from the P_i in the buffer could be used as a chemical shift reference. The pH of the buffer medium was measured immediately following the accumulation of the NMR spectra with a Corning Model 140 pH meter. Then the position of the buffer P_i peak was assigned the value calculated from the relation:

$$P_i = 3.22 + 2.51/(1 + 10^{6.803\text{-pH}})$$

derived by J. P. Phasmana et al. [3].

To measure the peak areas of the spectra of the kidneys accurately, they were deconvolved using a multivariant Simplex routine in which the height, width, and position of each peak is optimized by a least squared difference criterion. Furthermore, from the position of the intracellular P_i peak, the intracellular pH could be calculated using the inverse of the above equation.

Pretransplant MRS parameters and the intracellular renal pH were correlated with subsequent renal function in the animals, such as the drop in serum creatinine concentration, inulin renal clearances, and survival.

Electron microscopic analysis of variably ischemic renal tissue

A portion of the renal wedge biopsies used for HPLC analysis was subjected to EM study. Immediately upon removal of selected renal biopsy specimens, 1 mm thick shavings of renal cortex and medulla were dropped in to 5 ml of 0.1 *M* sodium cacodylate buffer (pH 7.4, 4 °C) and 3.75% glutaraldehyde containing 6% sucrose. After 24 h the tissue fragments were buffer rinsed and postfixed in cacodylate buffer with 1% osmium tetroxide for 1 h, then dehydrated in graded ethanols and embedded in spurr resin. Plastic sections (0.1 µm) were mounted on glass slides, stained with a mixture of toluidine blue and basic fuchsin, then examined under light microscopy. Representative blocks were selected, and corresponding thin sections were cut at 60–80 nm, stained in uranyl acetate and lead citrate, and examined in a Phillips 400 electron microscope. Endothelial cells and microcapillary anatomy were examined for evidence of ischemic reperfusion injury.

Table 2. Comparison of preservation solutions by survival post autorenal transplantation

	Survival	PB-2		C-2
Number of animals surviving	> 7 days	8/10	$P<0.01$	3/10
	>14 days	4/10	$P<0.01$	1/10
Mean Days Survival Post Transplant[a]		14±5	$P<0.02$	6±4

[a] Animals expiring secondary to pneumonia were excluded. Thus for PB-2, $n=6$ and for C-2, $n=7$

Table 3. Posttransplant mean serum creatinine concentration (mg/dl)

Days post-operation	0	1	2	3	4	5	6	7
PB-2	1.00	5.37	6.89	8.44	8.40	7.87	6.40	2.88
C-2	1.17	5.96	8.37	10.38	11.13	13.35	12.33	12.18

Table 4. Posttransplant Inulin clearance (ml/min)

	Preoperation		Postoperation
PB-2	37.6± 6.2	NS	42.9±33.8
C-2	51.3±15.6	$P<0.001$	14.6±16.0

NS, not significant

Table 5. HPLC study of canine renal biopsies

	PB-2[c]	C-2[c]	*P* value
TAN[a]			
In situ	6.54±2.33	5.32±1.50	NS
50 h cold storage	4.60±1.47	3.32±1.50	=0.01
5 min reperfusion	3.08±0.98	3.01±1.53	NS
45 min reperfusion	4.87±2.10	3.08±0.99	=0.02
DP[b]			
In situ	0.21±0.11	0.31±0.32	NS
50 h cold storage	3.06±1.19	1.01±0.78	<0.001
5 min reperfusion	0.27±0.21	0.23±0.08	NS
45 min reperfusion	0.25±0.19	0.41±0.16	<0.05

NS, not significant; [a] TAN (total adenine nucleotides) = ATP + ADP + AMP; [b] DP (degradation products) = hypoxanthine + xanthine; [c] All levels reported as μmoles/g dry wt renal cortical tissue

Statistical analysis

Categorical data were analyzed using Fisher's exact test or χ^2 depending upon expected cell frequencies. Univariate and multivariate, two-way, repeated measures ANOVA was utilized as appropriate to access differences due to treatment, time, and treatment by time interaction. After the determination of significant time effects by F tests, *t* tests were conducted to determine specific differences over time. The statistical analysis system (SAS) [10] was used to perform all statistical tests. A *P* value of less than 0.05 was used as the criterion for statistical significance. Where given, the results are expressed as the mean ± standard deviation.

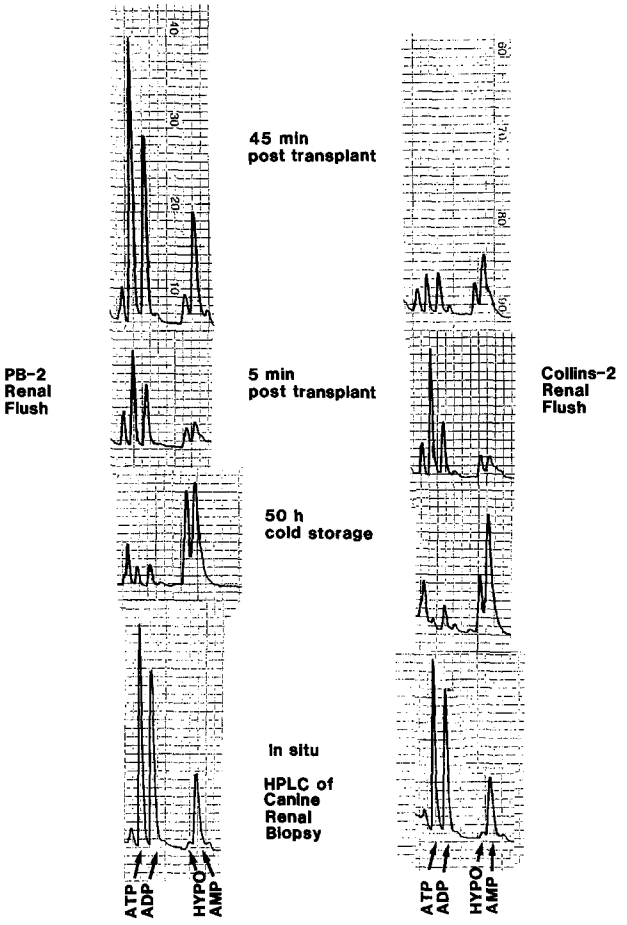

Fig. 3. HPLC study of canine kidneys. Note superior regeneration of adenine nucleotides by PB-2 during 5–45 min of reperfusion, while further deterioration during this time interval in the Collins-2 group is characteristic of "reperfusion injury"

Results

Recovery of renal function and survival

All autorenal transplants were technically successful with no vascular or urologic complications noted at autopsy. Deaths from pneumonia occurred randomly for each group, and these were excluded from statistical analysis of survival (Table 2) (PB-2 $n=4$; C-2 $n=3$). While immediate reperfusion and firmness was were noted in all kidneys after revascularization, the C-2 group had a palpable decrease in firmness after 45 min.

Animal survival was significantly greater in the PB-2 group as seen by the number of animals surviving ≥ 7 days and ≥ 14 days ($P<0.01$), as well as the mean number of days post renal transplantation ($P<0.02$) (Table 2).

The variable survival and condition of the animals posttransplantation limited complete monitoring of

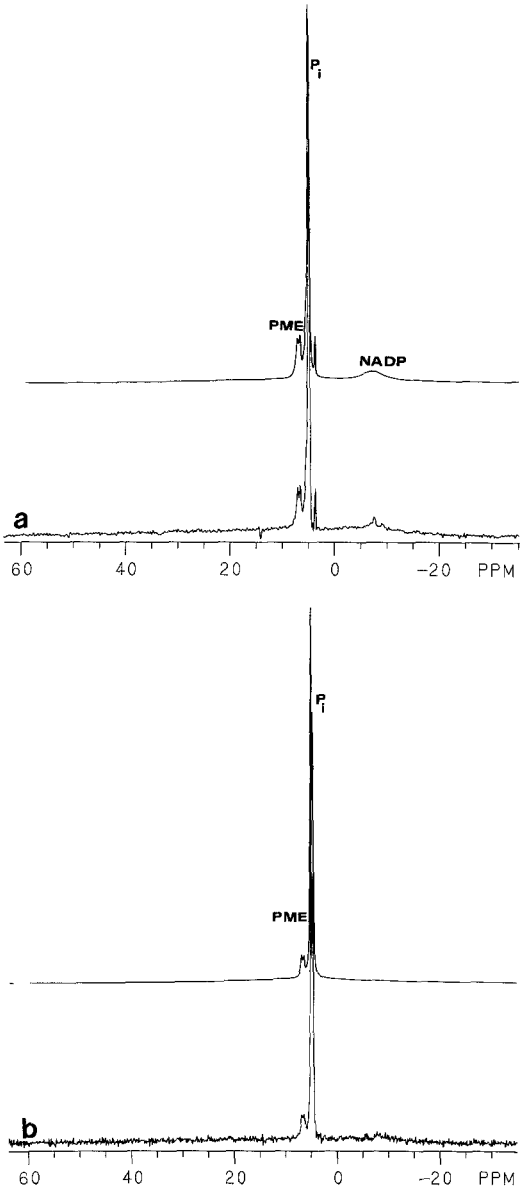

Fig. 4a, b. Representative ^{31}P-MRS spectra from whole canine kidneys at 50 h cold storage, just prior to autorenal transplantation. PB-2 flus (**a**) is associated with greater preservation of phosphomonoester (*PME*) (predominantly adenosine monophosphate for kidneys) [27] and nicotinamide-adenine-dinucleotide phosphate (*NADP*) compared with C-2 flush (**b**)

serum creatinine (Table 3) and inulin clearance (Table 4) for all animals. Nevertheless, both measurements reflected superior renal function recovery in the PB-2 group as compared with the C-2 group.

High performance liquid chromatography studies

Total adenine nucleotides (TAN) and adenine monophosphate (AMP) concentrations were significantly higher afte 50 h of cold storage in the PB-2 group as compared with the C-2 group (4.60 ± 1.47 vs 3.32 ± 1.50 μmole/g dry weight tissue, $P < 0.01$ and 3.30 ± 0.97 vs 2.42 ± 0.67 μmoles/g dry weight tissue, $P < 0.03$). TAN

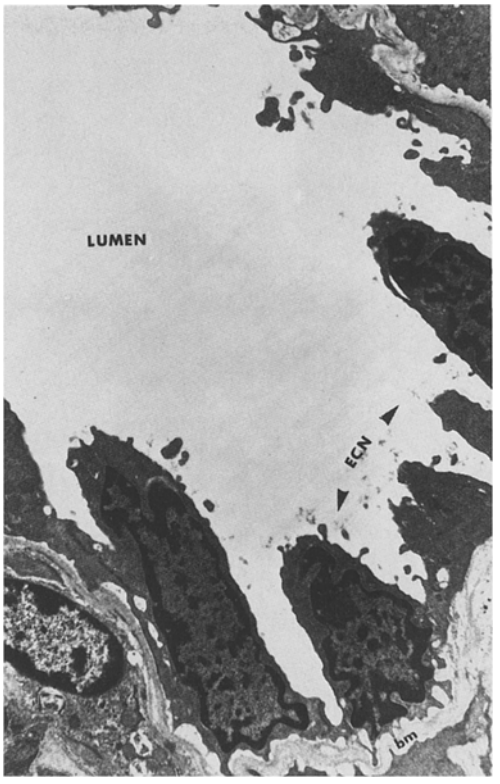

Fig. 5. Electron micrograph normal canine renal biopsies from kidneys cold stored with PB-2 for 50 h, then reperfused for 45 min. Note patent lumen, intact basement membrane (*bm*), and normal endothelial cell (*ECN*) nuclei morphology. ×10,320

were significantly ($P < 0.03$) higher and degradation products (DP) significantly ($P < 0.05$) lower [despite having had significantly ($P < 0.001$) higher levels after cold storage] in the PB-2 group after 45 min of reperfusion compared with the C-2 group (Table 5). Figure 3 demonstrates a typical comparison of the regeneration of AMP, ADP, and ATP by PB-2 compared with C-2. While no significant difference was noted at 5 min of reperfusion, a variation was observed by 45 min. Further deterioration of TAN noted during the interval between 5 and 45 min within the C-2 group is consistent with postreperfusion injury.

Magnetic resonance spectroscopy studies

Levels of high energy phosphorus metabolites were greater in the PB-2 group compared with the C-2 group just prior to transplantation (PME/P_i: 0.41 ± 0.26 vs 0.26 ± 0.09, $P < 0.10$ and $NADP/P_i$: 0.36 ± 0.41 vs 0.01 ± 0.03, $P < 0.0001$); however, no significant difference in intracellular pH was noted (7.13 vs 7.20). Representative spectra illustrate these major differences of renal intracellular metabolites (Fig. 4a, b).

Electron microscope studies

EM analysis revealed normal endothelial cell (ECN) morphology and patent microcapillary lumens from con-

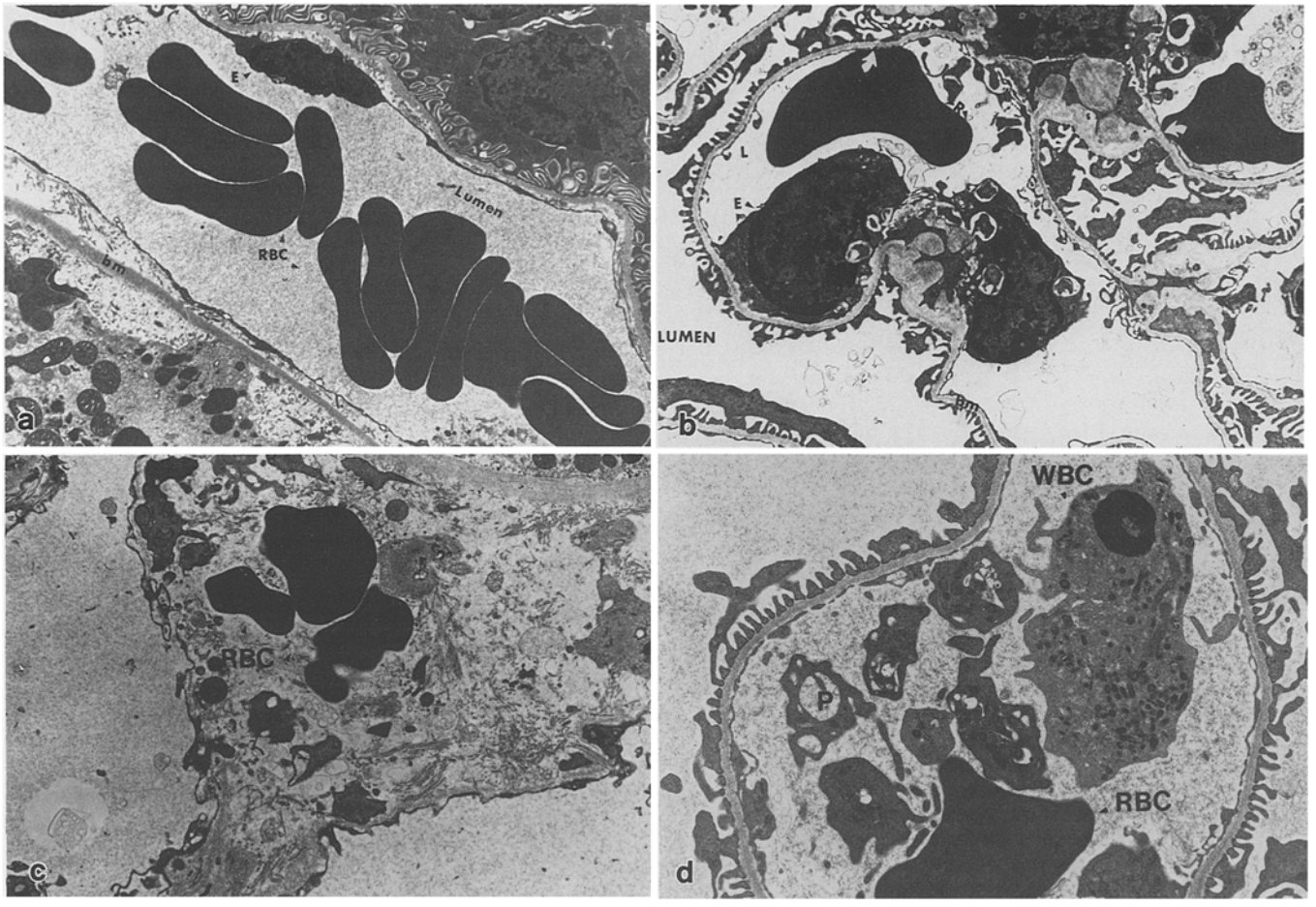

Fig. 6a–d. Electron micrographs of 45 min postreperfusion biopsy of 50 h cold stored C-2-flushed canine kidney. Note: red blood cell (RBC-RBC; **a**) and endothelial cell (RBC-ECN; **b**) adhesiveness (*arrows*), as well as RBCs within the interstitial spaces (**c**). In addition, earlier biopsies at 5 min postreperfusion show a greater presence of white blood cells (*WBC*) RBC and platelets (*P*) aggregating within the capillary lumen (**d**). These abnormalities are characteristic of "postreperfusion injury" and were not associated with kidneys preserved with PB-2 solution. ×6,960

trol in situ biopsies of normal kidneys in both groups. However, while PB-2-flushed kidneys at 5 min (Fig. 5) and at 45 min postreperfusion were also normal, the biopsies from C-2-flushed kidneys at these intervals were associated with red blood cell (RBC) clumping within the microcapillary lumen via RBC-RBC (Fig. 6a) and RBC-ECN (Fig. 6b) adhesiveness. In addition, interstitial microhemorrhage (Fig. 6c) and aggregation of leucocytes (WBCs), RBCs, and platelets within the capillary lumen were frequently noted (Fig. 6d). These changes were progressive during the 5–45 min reperfusion period.

Discussion

High performance liquid chromatography results

Studies using HPLC [26], ^{31}P-MRS [1, 5–8], and EM [28] have helped to elucidate cellular processes integral to successful kidney preservation. Higher AMP levels, as demonstrated by HPLC, correspond directly with higher levels of PME, as measured by MRS, at 50 h of cold storage. These findings are consistent with other recent observations showing that the PME peak may be predominately composed of AMP [1]. HPLC and ^{31}P-MRS are demonstrated to be complementary methods which can identify different intracellular metabolites necessary for tissue viability.

The higher HPLC measured levels of DP at 50 h of cold storage should predict a more severe reperfusion injury, since DP are associated with the formation of toxic free radicals. However, this was not observed (Table 5), possibly reflecting the efficiency of mannitol (a free radical scavenger) in the PB-2 solution in minimizing this effect.

Magnetic resonance spectroscopy results

In the present study, ^{31}P-MRS measurements at 50 h of cold storage, just prior to renal transplantation, revealed greater levels of PME (predominately AMP, $P < 0.10$) and NADP ($P < 0.05$). No significant difference in intracellular renal pH was noted between the groups. These findings are consistent with recent observations in human kidneys studied by MRS demonstrating the ability of this method to assess renal viability accurately during cold storage [8]. In addition, the current study shows a trend to greater preservation of high energy intracellular phosphorus

metabolites with PB-2 as compared with C-2. This mechanism may be completely separate from those which alleviate reperfusion injury, and the use of adenosine in PB-2 solution may account for this by retarding ischemic catabolism of AMP and adenosine (Fig. 2) through feedback inhibition of the responsible enzymes.

Electron microscopy results

The current EM studies further define morphologic criteria for reperfusion injury following cold preservation. We noted an increase in RBC, WBC, and platelet aggregation in the microcapillaries of the kidney. This observation has been supported by studies using ^{51}Cr-labelled erythrocytes. For example, Jacobsson and coworkers [22] have shown that reperfusion following cold ischemia in the rat kidney causes a significant increase in RBC trapping. This effect may be prevented through the use of free radical scavengers (FRS), such as superoxide dismutase or allopurinol. Thus, the reperfusion injury appears to be mediated by oxygen-derived free radicals (FR), such as superoxide (O_2^-) and hydroxyl ion (OH^-) (Fig. 1). These FR can disrupt cell membranes, resulting in severe vascular endothelial cell damage during the reperfusion period, contributing to further surrounding tissue damage (Figs. 1, 2). During cold storage, ischemia causes extensive catabolism of ATP and other ANs, resulting in the accumulation of tissue HYPO (Fig. 2). The latter is a substrate for xanthine oxidase, which generates high levels of FR during reperfusion. Extensive ATP catabolism from ischemia in itself is a major component of irreversible tissue injury. The ischemic (catabolic derived) and reperfusion (FR derived) components are the two major contributors to total postischemic renal injury, as supported by the recent studies of Hoshino and coworkers [20]. Both of these mechanisms must be adequately controlled in order to enhance renal preservation.

Rationale for PB-2 composition

PB-2 flush solution (Table 1) was specifically designed to minimize both ischemic processes (catabolic and reperfusion) as well as their effects. Mannitol has multiple modes of action in renal preservation. For example, it is an effective FRS for OH^- [33] (Fig. 2), a detoxifier [32], and an indispensable constituent of intraoperative hydration protocols for the prevention of acute renal failure after renal cadaveric transplantation [37]. On the other hand, dextrose, which is currently used for its hyperosmolar effect in the C-2 flush solution, has been shown to exacerbate acute renal ischemic damage in dogs [27]. Thus, mannitol has been substituted for dextrose in the PB-2 solution.

Adenosine [2, 36] and magnesium [12, 13] were added to the PB-2 solution because they have been shown to improve the postreperfusion microcirculation and to enhance the maintenance and regeneration of intracellular ATP and AN. Magnesium also exhibits a vasodilator effect and acts as a metabolic inhibitor [12].

The overall composition of PB-2 (Table 1) differs from the C-2, Belzer perfusate, and UW-1 flush solutions. Good results with UW-1 solution have recently been reported [39, 41]; however, concerns over its high cost and the possibility of an allergic reaction to the high concentration of hydroxyethyl starch (50 g/l) have slowed widespread usage.

Sacks I (S-1) and 2 (S-2) flush solutions, which similarly use mannitol, also have an intracellular composition and are hyperosmolar [35]. However, there are many studies that show inferior preservation when compared with C-2 solution [12, 14], as well as failure to achieve 48 h of safe preservation adequately [11]. The basic difference that may account for this, is the lack of magnesium and adenosine in S-1 solution. S-2 solution also lacks adenosine and may have an inferior buffering capacity as reflected by its greater acidity (pH = 7,0) compared with that of PB-2 (pH = 7.25), as measured using the WESCOR 5100B vapor pressure osmometer). In addition, the greater hyperosmolarity of the Sacks mixtures (410–430 mOsm/kg) may greatly increase the tendency of mannitol and magnesium phosphate to precipitate out of solution within the kidney during cold storage [12].

Rationale for PB-3 composition

There is a growing experience that supports the use of other additives, such as enzyme inhibitors, FRS, buffers, and calcium entry blockers (CEB), for enhanced organ preservation. For example, the use of allopurinol [10] to slow the generation of oxygen FR at reperfusion has been successful, as well as the use of histidine for a higher pH buffering capacity [9]. Also 2′-deoxycoformycin has been used to increase the residual AN pool by inhibition of 5′-nucleotidase, the enzyme responsible for the breakdown of high energy ANs to HYPO during ischemia [38]. Lastly, the use of CEB for both donor pretreatment [21, 24]. and as a cold flush additive [29], is gaining widespread support. CEB are known to block cell membrane calcium channels. This is an important step in the prevention of a cascade of intracellular responses that amplify the postischemic reperfusion injury. For example, during postischemic reperfusion, influx of extracellular Ca causes a sudden increase in cytosolic Ca (Ca_i). A high level of Ca_i activates cellular phopholipases, proteinases, and its own accumulaton in large amounts in the mitochondria. By preventing the subsequent Ca-dependent enzyme activation of phospholipases (preventing subsequent membrane degradation) (Fig. 2.), xanthine dehydrogenase (XD) activation is also decreased, preventing activation of xanthine oxidase (XO), responsible for the subsequent FR generation burst during reperfusion. In addition, CEB can prevent excessive Ca accumulation in the mitochondria. Without CEB, subsequent high levels of mitochondrial Ca will uncouple oxidative phosphorylation, and hence rephosphorylation of ADP to ATP is abolished. As reperfusion progresses, further deterioration of vital cell processes will arise due to inadequate regeneration of ATP, ultimately ending in cell death.

Because of these recent findings, we propose the addition of allopurinol, verapamil, and added buffer (to increase pH above 7.40) to PB-2 solution (Table 1, PB-3 flush) for further enhancement of preservation. Further studies are required to investigate the efficacy of these

contentions, but the current one has suggested that PB-2 renal flush significantly enhances simple hypothermic renal preservation by reducing the reperfusion injury not alleviated by C-2 solution and better maintenance of intracellular high energy metabolites required for subsequent viability.

References

1. Baldwin N, Bretan PN, Scarpa A (1988) Predominance of renal phosphomonoester (PME) ^{31}P-MRS peak by AMP – correlative and quantitative analysis using HPLC assay. Proceedings of Society of Magnetic Resonance in Medicine (works in progress suppl) p 77
2. Belzer FO, Sollinger HW, Glass NR, et al. (1983) Beneficial effects of adenosine and phosphate in kidney preservation. Transplantation 36:633
3. Belzer FO, Hoffman RM, Miller DT, et al. (1984) A new perfusate for kidney preservation. Transplant Proc 16:161
4. Bretan PN (1989) Extracorporeal renal preservation. In: Novick AC (ed) Stewart's textbook of operative urology. Williams & Wilkins, Baltimore
5. Bretan PN, Vigneron DG, James TL, Williams RD, Hricak H, Juenemann KP, Yen B, Tanagho EA (1986) Assessment of renal viability by phosphorus 31 magnetic resonance spectroscopy. J Urol 135:866
6. Bretan PN, Vigneron DB, Hricak H, Juenemann KP, Tanagho EA, James TL (1986) Assessment of renal preservation by ^{31}P-MRS: in vivo normothermic blood perfusion. J Urol 136:1356
7. Bretan PN Jr, Vigneron DB, Hricak H, Price D, Collins GM, Tanagho EA, James TL (1987) Assessment of clinical renal preservation by phosphorus-31 magnetic resoncance spectrocopy. J Urol 137:146
8. Bretan PN, Baldwin N, Novick AC, et al. (1989) Pretransplant assessment of renal viability by ^{31}P-MRS. Transplantation 48:48
9. Busza AC, Fuller BJ, Protor E, Gadian DG (1988) Improved maintenance of tissue phosphorus metabolites and control of tissue Ph as demonstrated by ^{31}P-MRS in rat livers flushed-stored at 4°C using a histidine-buffered citrate solution. Proc Soc Mag Reson Med, San Francisco, p 465
10. Cary NC (1965) SAS user's guide – statistics, 5 ed. SAS Institute, p 956
11. Chatterjee SN, Berne TV (1975) Failure of 48 hours of cold storage of canine kidneys using Sacks solution. Transplantation 19:441
12. Collins GM (1983) Current status of renal preservation by simple flushing and hypothermic storage. In: Marberger M, Dreikorn K (eds) Renal preservation. Williams & Wilkins, Baltimore, Chap. 15, p 224
13. Collins GM, Halasz NA (1976) 48 hour ice storage of kidneys – importance of cation content. Surgery 79:432
14. Dahlager JI, Bilde T (1976) The integrity of tubular cell function after preservation in Collins or Sacks solution. Transplantation 21:365
15. Davidson WD, Sackner MA (1963) Simplification of anthrone method for the determination of inulin clearance studies. J Lab Clin Med 62:351
16. Erturk E, Bretan PN, Pestana J, et al. (1989) The relative effects of aztreonam vs. gentamycin on nephrotoxicity induced by warm ischemia in the presence and absence of cyclosporine. Transpl Proc 21:932
17. Maessen JG, Greve J, Buurman WA (1989) Sensitivity of ischemically damaged kidney to inflammatory reactions. Transpl Proc 21:1261
18. Jamieson NV, Lindell S, Sundberg R, Southard JH, Belzer FO (1989) Evaluation of simplified variants of the UW solution using the isolated perfused rabbit liver. Transplant Proc 21:1294
19. Henry ML, Sommer BG, Ferguson RM (1988) Improved immediate function of renal allografts with Belzer perfusate. Transplantation 45:73
20. Hoshino T, Maley WR, Bulkley GB, Williams GM (1988) Ablation of free radical-mediated reperfusion injury for the salvage of kidneys taken from non-heart-beating donors – a quantitative evaluation of the proportion of injury caused by reperfusion following periods of warm, cold, and combined ischemia. Transplantation 45:284
21. Ishigami M, Magnusson M, Stowe N, Straffon R (1984) The salutary effect of verapamil and propranolol in ischemically damaged kidneys. Transplant Proc 16:40
22. Jacobsson J, Odlind B, Tufveson G, Wahlberg J (1988) Effects of cold ischemia and reperfusion on the trapping of erythrocytes in the rat kidney. Transplant Int 1:75
23. Keller H, Fischer G, Kirste G, Wilms H (1989) ATN influence on renal transplant function. Transpl Proc 21:1282
24. Korb SM, Albornoz W, Brem A (1989) Verapamil pretreatment of hemodynamically unstable donors prevents acute tubular necrosis post-transplant. Transplant Proc 21:1236
25. Koyama I, Bulkley GB, Williams GM, Im MJ (1985) The role of oxygen free radicals in mediating the reperfusion injury of cold-preserved ischemic kidneys. Transplantation 40:590
26. Maessen JG, van der Vusse GJ, Vork M, et al. (1988) Determination of warm ischemia time at donor nephrectomy. Transplantation 45:147
27. Moursi M, Rising CL, et al. (1987) Dextrose administration exacerbates acute renal ischemic damage in anesthetized dogs. Arch Surg 122:790
28. Neil D, Pollock GA, Molyneux GS, Hardie IR (1989) Vascular changes following hypothermic perfusion. Transplant Proc 21:1389
29. Neumayer HH, Schreiber M, Wagner K (1989) Prevention of delay graft function in cadaveric kidney transplants by calcium antagonist diltiazem and prostacyclin-analogue iloprost – outcome of a prospective randomized clinical trial. Transplant Proc 21:1221
30. Novick AC, Ho-Hsieh H, Steinmuller D, Streem SB, et al. (1986) Detrimental effect of cyclosporine on initial function of cadaver renal allografts following extended preservation – results of a randomized prospective study. Transplantation 42:154
31. Opelz G (1986) Multicenter impact of cyclosporin on cadaver kidney graft survival. Prog Allergy 38:329
32. Palafox NA, Luis GJ, Alexander Z, et al. (1988) Successful treatment of ciguatera fish poisoning with intravenous mannitol. JAMA 259:2740
33. Pavlock GS, Southard JH, Lutz M, et al. (1981) Effects of mannitol and chlorpromazine pretreatment of rabbits on kidney mitochondria following in vivo ischemia and reflow. Life Sci 29:2667
34. Phasmana JP, Digerness SB, Ng TC, Glickson JD, Blackstone EH (1983) The effect of adenosine deaminase inhibitors on the hearts functional and biochemical recovery from ischemia. J Cardiovasc Pharmacol 5:1040
35. Sacks SA, Petritsch PH, Kaufman JJ (1973) Canine kidney preservation using a new perfusate. Lancet I:1024
36. Stromski ME, Cooper K, Thulin G, et al. (1986) Post ischemic ATP-Mg Cl_2 provides precursors for resynthesis of cellular ATP in rats. AM J Physiol 250:F834
37. Valenberg PL, Hoitsma AJ, Tiggeler RG, et al. (1987) Mannitol as an indispensable constituent of an intra-operative hydration protocol for the prevention of acute renal failure after renal cadaveric transplantation. Transplantation 44:784
38. Van Waande A, Stromski ME, Thulin KM, et al. (1988) Protection of kidney against ischemic injury by inhibition of 5′-nucleotidase. Proc Soc Mag Reson Med, San Francisco, p 628
39. Wahlberg JA, Love R, Landegaard L, et al. (1987) 72 hour preservation of the canine pancreas. Transplantation 43:5
40. Wynants J, Van Belle H (1985) Single-run high-performance liquid chromatography of nucleotides, nucleosides, and major purine bases and its application to different tissue extracts. Anal Biochem 144:258
41. Ploeg RJ, Goossens D, Vreugdenhil P, et al. (1988) Successful 72 hour storage of dog kidneys with UW solution. Transplant Proc 20 [Suppl I]:935

Peter N. Bretan Jr., MD
Department of Urology
Harbor UCLA Medical Center
1000-West Carson
Torrance, California 90509, USA