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Characterization of improved renal transplant preservation mechanisms using PB-2 flush solution by HPLC assay

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Abstract Renal flush solutions have been found to be beneficial for extending organ viability, but their mechanisms of action are poorly understood. In order to delineate these mechanisms we studied the addition of mannitol and adenosine to a modified simple hypothermic intracellular flush solution (PB-2), and the relationships of renal adenine nucleotide (AN) concentrations with ischemia, reperfusion and viability. A significant ($P < 0.05$) and progressive decay in AN and increase in total degradation products (DP; hypoxanthine and xanthine) was noted during warm ischemia. Total AN and AMP were significantly higher after 50 h of cold storage in the PB-2 compared to the C-2 control cold flush group. Viability was associated with a significant ($P < 0.01$) regeneration of AN within 45 min of reperfusion. HPLC assay indicated that PB-2 cold flush solution enhances renal viability by both diminution of reperfusion injury enabling better reflow and primary preservation of AN. The latter process may appreciably contribute to the former process.

Key words Renal transplantation · Organ preservation · Intracellular flush solution · High performance liquid chromatography

Introduction

A number of additives to renal flush solutions have been found to be beneficial for extending transplant organ viability during simple cold storage. However, the mechanisms, as well as the relative importance of each of those preservatives remain poorly understood. We have previously presented a modified intracellular flush solution (PB-2) that was found to be superior to Collins (C-2) solution during cold storage [1]. Graft survival was significantly greater ($P < 0.01$) and recovery of renal function significantly faster compared to the C-2 group. PB-2 consists of an intracellular solution (340 osm/kg) with the addition of adenosine and mannitol. In summary, this previous study has shown that the mechanisms involved include both diminution of reperfusion injury and maintenance of intracellular high energy metabolites. The purpose of the present study was to further delineate, using HPLC assays, the relationships of renal adenine nucleotide (AN) concentrations with warm and cold ischemia, reperfusion, and viability.

Materials and methods

Serial biopsies were taken from canine kidneys ($n = 25$) during warm ischemia (0–120 min), cold storage (0–50 h), and subsequent reperfusion (5–45 min). In the latter two groups, PB-2 was compared to C-2 solution. The composition of each solution has been previously

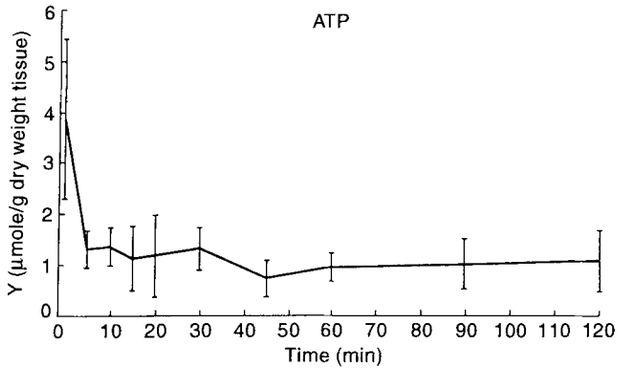

Fig. 1 HPLC assay of canine kidneys: rapid decay of ATP in 1st 8 min of warm ischemia

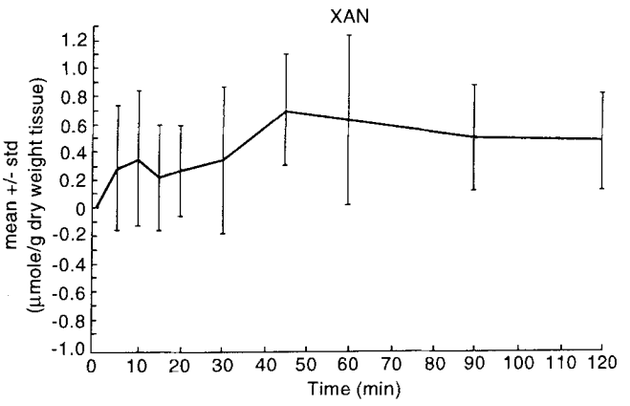

Fig. 4 HPLC assay of canine kidneys: increase in xanthine (XAN) during 1st 35 minutes of warm ischemia

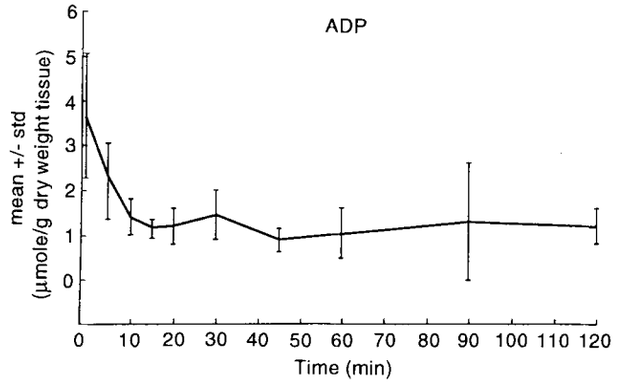

Fig. 2 HPLC assay of canine kidneys: rapid decay of ADP in 1st 15 min of warm ischemia

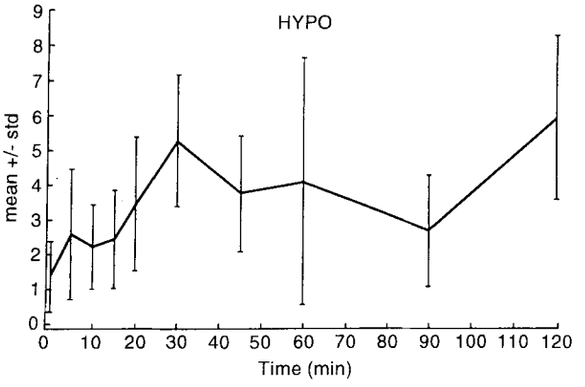

Fig. 5 HPLC assay of canine kidneys: increase in hypoxanthine (HYPO) during 1st 35 min of warm ischemia

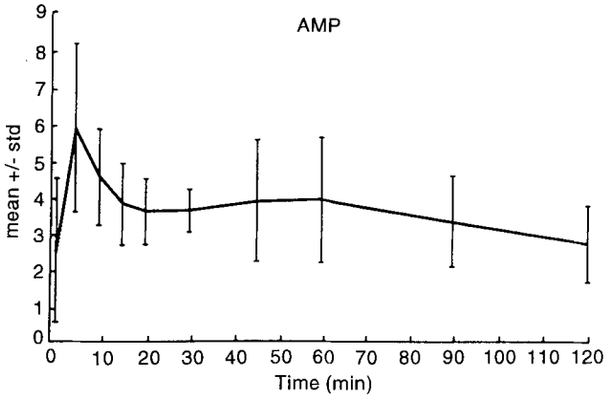

Fig. 3 HPLC assay of canine kidneys: rapid increase in AMP in 1st 10 min, then subsequent decay during 2nd 10 min of warm ischemia

described [1]. ANs were extracted from freezed-clamped renal specimens and analyzed using reverse phase high performance liquid chromatography (HLPC) with a Waters instrument, as previously described [1, 2].

Results

Good resolution was obtained with all major ANs (ATP, ADP, and AMP), as well as their degradation products (DP; hypoxanthine and xanthine). ATP (Fig. 1) and ADP (Fig. 2) levels dropped rapidly to baseline during the first 10 min of warm ischemia. During this same time period, AMP levels rose to a maximum (Fig. 3). Xanthine (XAN; Fig. 4) and hypoxanthine (HYPO; Fig. 5) rose to a maximum at 35 min of warm ischemia. A significant ($P < 0.05$) and progressive decay in AN (Fig. 6) and

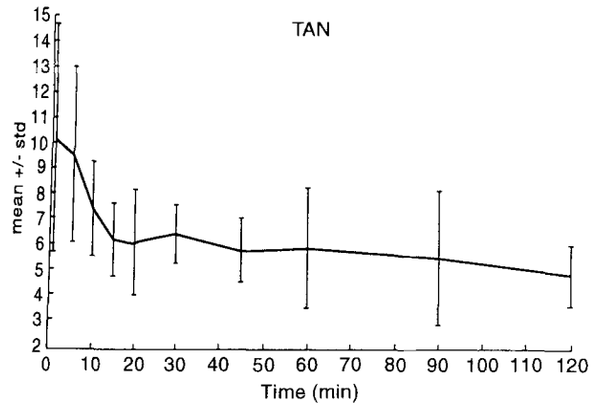

Fig. 6 HPLC assay of canine kidneys: decay of TAN (total adenine nucleotide) during warm ischemia

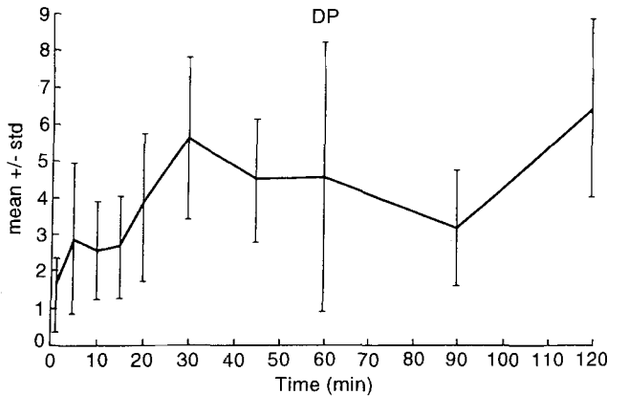

Fig. 7 HPLC assay of canine kidneys: increase in DP (total degradation products, xanthine and hypoxanthine) during warm ischemia

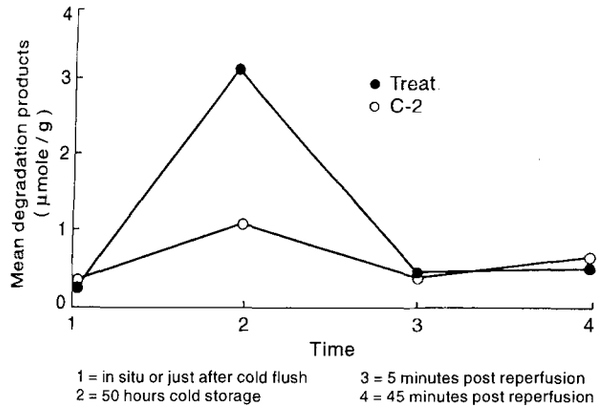

Fig. 8 HPLC assay of canine kidneys. TREAT = PB-2 flush cold stored group

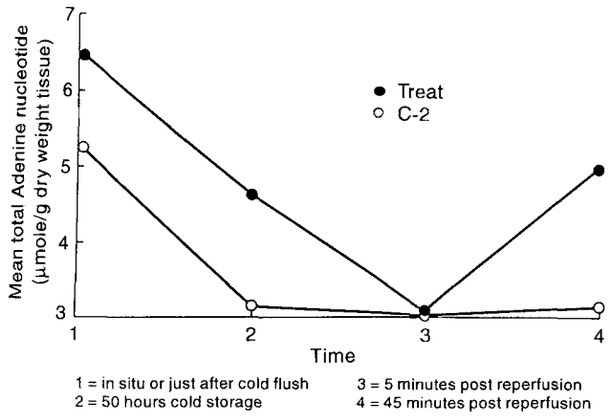

Fig. 9 HPLC assay of canine kidneys. TREAT = PB-2 flush cold stored group

steady increase in DP (Fig. 7) was noted with increasing warm ischemia (0–30 minutes) time.

Total adenine nucleotides (TAN, ATP + ADP + AMP); (Fig. 6) and AMP were significantly higher after 50 h of cold storage in the PB-2 ($n = 10$) group compared with a C-2 control ($n = 10$) group (4.60 ± 1.47 vs. 3.32 ± 1.50 µmol/gram dry weight tissue, $P < 0.01$ and 3.30 ± 0.97 vs. 2.42 ± 0.67 µmol/gram dry weight tissue, $P < 0.03$). Despite having had significantly ($P < 0.001$) higher DP levels after cold storage in the PB-2 (treated) group (Fig. 8), TAN (Fig. 9) were significantly ($P < 0.03$) higher and DP (Fig. 8) significantly ($P < 0.05$) lower in the PB-2 group after 45 min of reperfusion compared to the C-2 group. Overall, less reperfusion injury was noted in the PB-2 group compared to the C-2 group (Fig. 10; from reference [1]).

Discussion

The mean TAN drops with ischemia and higher levels seem to be a good prognostic indicator for better renal preservation and subsequent sustained reperfusion. The addition of adenosine to PB-2 cold flush may contribute to this observation by two mechanisms:

1. A slowing of catabolism
2. An increase in substrate provisions during reoxygenation

Thus, adenosine may preserve renal cellular energy charge (ATP/ADP), or slow glycolysis via conformational changes in associated enzymes such as phosphofructokinase and other intramembranous proteins

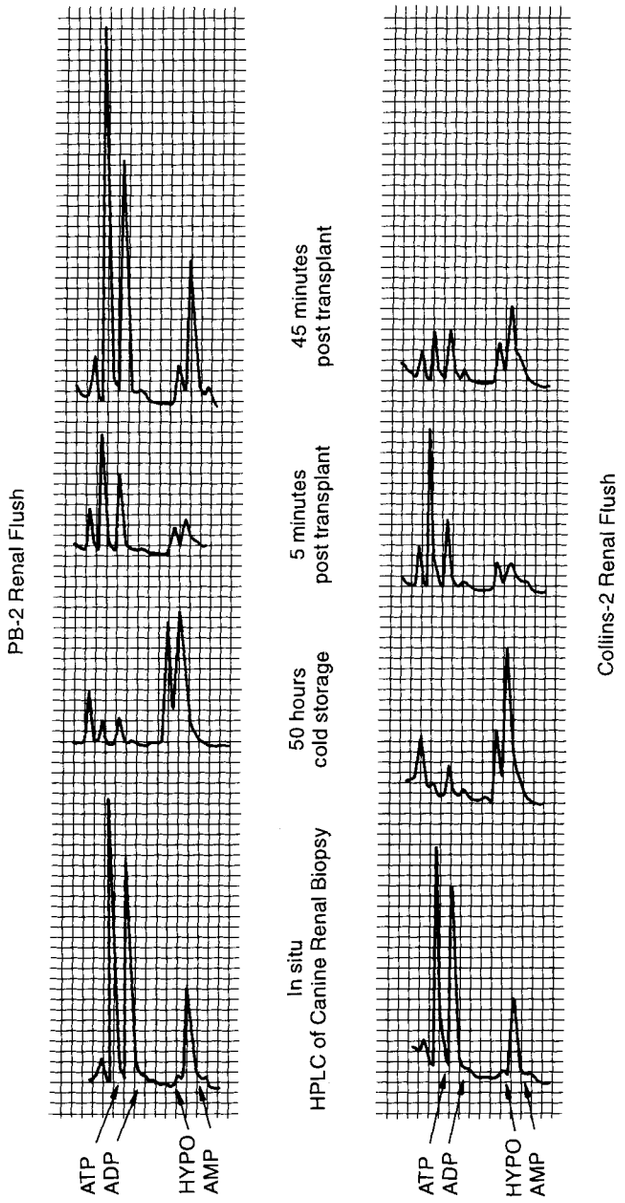

Fig. 10 HPLC assay of canine kidneys. From reference [1]. Note deterioration of adenine nucleotide (AN) in C-2 cold storage group during reflow period, consistent with reperfusion injury. In PB-2 group superior regeneration of AN is noted during the same 5- to 45-min reperfusion period

used in oxidative phosphorylation, thus slowing global cellular metabolism and needs.

On the other hand, an increase in DP was seen after cold storage in the PB-2-treated group suggesting that the adenosine addition may lead to greater generation of free radicals (FR). Nevertheless, better preservation was noted in the PB-2 group as well as less reperfusion injury. The presence of mannitol in PB-2 may account for efficient scanvenging of the hydroyl FR as we have previously postulated [1], thus, effectively counteracting the generation of the FR that are associated with increase levels of DP, and reperfusion injury. This suggests that these two events, AN preservation and prevention of reperfusion injury are closely linked and are not completely independent processes. Thus, preservation maneuvers must act appropriately in dealing with both of these mechanisms in order to be successful.

In summary, viability was associated with a significant ($P < 0.01$) regeneration of AN within 45 min of reperfusion. Renal "reperfusion injury" was characterized by immediate (at 5 min) regeneration of AN, followed by subsequent loss of AN (at 45 min). Characterization of the PB-2 solution's basic preservation mechanisms by HPLC assay indicated that it enhances renal viability by both: (1) diminution of a "reperfusion injury", enabling better reflow and (2) primary preservation of, as well as immediate regeneration of AN, which may appreciably contribute to process (1).

References

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2. Wynants J, Van Belle H (1985) Single-run HPLC of nucleotides, nucleosides, and major purine bases and its application to different tissue extracts. Anal Biochem 144:258