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A new intracellular flush solution improves renal transplant preservation

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■ A new renal preservation flush solution (PB-2) has been developed to minimize the ischemic injury processes that occur during hypothermic storage and reperfusion and that can decrease renal viability and survival. Development of the new formulation took into account the intracellular and biological interactions that occur pre- and post-transplantation. PB-2 was compared with the conventional standardized Collins-2 flush solution in the preservation of autografts in dogs and was found to provide significantly improved renal recovery, viability, and survival. Studies to test the new solution in human renal allograft transplant preservation are planned.

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KIDNEY transplantation is now almost a routine. Both related-donor and cadaveric transplants are performed at a rate limited only by the donor supply.

Much of the current literature on transplantation concerns immunosuppression after the transplant is performed. With such powerful agents as cyclosporine and OKT3 available, some centers advocate performing only cadaveric transplants. Because the time between harvesting the kidney and transplantation is often (necessarily) prolonged, the methods of kidney preservation during this time have an important role in the transplantation process.

The accompanying article by Bretan and Novick, part of the "Bench to Bedside" series, describes a new perfusion solution that could improve survival of the renal allograft. Based on knowledge of the intracellular processes that occur during storage and reperfusion, the authors created a solution that effectively preserves cellular, and thus total renal, function. — B.H.B.

The preservation of kidneys between harvest and transplantation involves simple cold storage with standardized flush solutions. Significant acute tubular necrosis can occur after 24 hours, which is the mean storage time documented at the Cleveland Clinic. Intracellular molecular processes that occur during the storage and reperfusion periods may be either beneficial or detrimental to renal viability. Recent research has improved our understanding of these processes and given us rationales for manipulating the components of the standard cold renal preservation flush solution. The result is a new solution that will be evaluated in clinical trials.

Since the beginning of the cyclosporin A (CSA) era in renal transplantation, organ preservation has been an important issue. With the use of CSA, a synergistic nephrotoxic effect has been noted prior to transplantation when a donor kidney sustains a significant ischemic insult, such as prolonged preservation.[1–3] There is increasing evidence that moderate ischemic injury

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may predispose the renal allograft to severe rejection and diminished survival.[1,4–6] Preservation is even more important for nonrenal organs, such as the pancreas, liver, and heart. As with kidneys, improved methods of organ preservation would facilitate both regional and national organ transplant.

The intracellular mechanisms that are integral to successful organ preservation (*Figures 1* and *2*)[6] have been identified through the use of such research tools as phosphorus 31 magnetic resonance spectroscopy (^{31}P-MRS),[7–9] electron microscopy,[7,10] and high-performance liquid chromatography (HPLC).[11,12] Studies have indicated that organ preservation methods are important during cold storage and during the immediate post-transplant reperfusion period.[13,14] It is postulated that preventable and reversible injury occurs at the intracellular metabolic level at these time periods (*Figures 1* and *2*). Since the composition of renal preservation flushing solutions may alleviate or prevent cellular injury during the reperfusion period, the development of new, improved solutions is warranted.

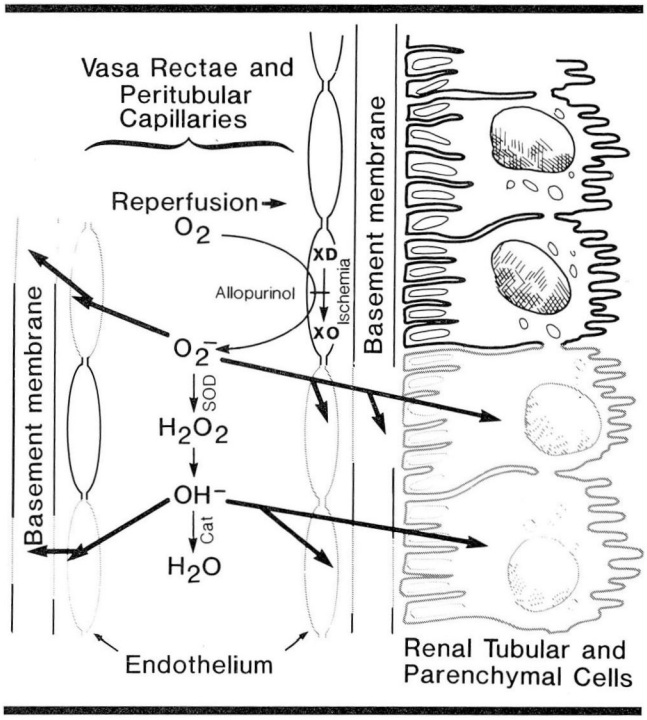

FIGURE 1. Mechanism of renal reperfusion injury after transplant revascularization. O_2–, superoxide; OH–, hydroxyl ion; XD, xanthine dehydrogenase; XO, xanthine oxidase; SOD, superoxide dismutase; CAT, catalase (from Bretan6).

MANEUVERS FOR PRESERVATION

To understand the rationales for renal preservation techniques, we must examine specific cellular events that occur during organ harvest warm ischemia and subsequent hypothermic storage and reperfusion. Four basic maneuvers are employed in renal preservation to counteract specific cellular pathologic processes: (1) hypothermia, (2) intracellular flush solutions, (3) preservation of intracellular high energy metabolites, and (4) use of free radical scavengers (FRS).

Hypothermia (4°C) during the ex situ period slows metabolic activity by a factor of 10 to 20 and greatly diminishes loss of energy stores and damage from lack of oxygen and blood flow. Cellular swelling occurs during this period because the sodium, potassium, and calcium pumps of the cell membrane shut down at 4°C. The intracellular environment consists of high concentrations of potassium, phosphate, and protein compared to the extracellular space; to counteract the tendency for water to enter the cells, solutions with high potassium and phosphate levels and high osmolality (intracellular components) are used to flush and store organs during the hypothermic period.

Endothelial cell damage is a primary ischemic injury that occurs after revascularization. Additives such as adenosine and FRS may diminish this damage.

During cold ischemia (*Figure 2*), adenosine triphosphate (ATP) is degraded to hypoxanthine. Both hypothermia and adenosine can slow this process. In addition, xanthine dehydrogenase (XD) is converted to xanthine oxidase (XO). XO in turn degrades hypoxanthine to xanthine, which is coupled to superoxide (O_2–) generation.

Allopurinol can inhibit XO, thus diminishing O_2– generation. In the presence of a sudden introduction of O_2 after significant hypoxanthine degradation, as is seen during reperfusion of transplant organs, the normally abundant free radical scavengers, such as superoxide dismutase (SOD) and catalase (CAT), are functionally depleted and O_2– accumulates. Once enough O_2– is generated (*Figures 1 and 2*), a series of reactions can lead to hydroxyl ion (OH–) generation.

Both O_2– and OH– can cause lipid peroxidation and directly damage membranes of the vascular endothelial cells comprising the lining of the vasa rectae and peritubular capillaries in the kidney, ultimately causing small vessel thromboses. This can lead to secondary ischemic damage to renal tubular and parenchymal cells. Thus, the primary injury is not a whole organ injury, but an endothelial injury which occurs at the

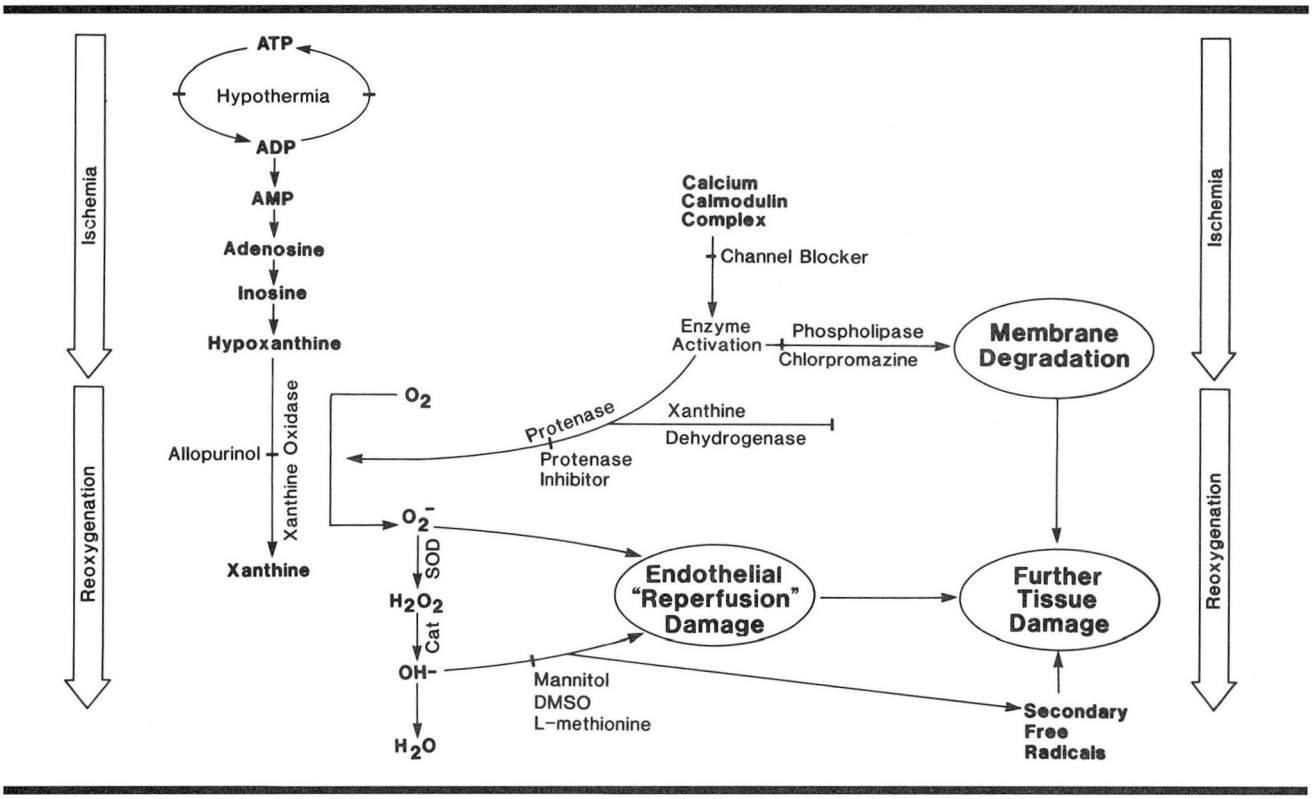

FIGURE 2. Proposed metabolic pathways involved in renal transplant preservation. O_2–, superoxide; OH–, hydroxyl ion; SOD, superoxide dismutate; CAT, catalase; DMSO, dimethyl sulfoxide (from Bretan[6]).

time of reperfusion.

OH– is much more toxic than O_2–; however, OH– can be deactivated by CAT, mannitol,[15] dimethyl sulfoxide, and L-methionine. Further cell membrane damage is triggered by activation of calcium-dependent phospholipase (*Figure 2*), which can be blocked by calcium entry blockers, calmodulin inhibitors, or chlorpromazine.

Collins solution (Collins-2) is an intracellular cold flush preservative, high in potassium and phosphate (*Table 1*). It has been used as the standard for simple hypothermic storage of kidneys for clinical transplantation for the past 20 years. We modified its composition based on an understanding of the processes just discussed and on the following rationales.

RATIONALES FOR THE COMPOSITION OF PB-2

Development of the new organ preservation solution (PB-2) has taken into account many intracellular and biochemical interactions. PB-2 flush solution (*Table 1*) was specifically designed to minimize both ischemic injury processes (catabolic and reperfusion), and the consequences of such injury.

In the PB-2 solution, mannitol has been substituted for glucose in the Collins-2 solution. Mannitol has multiple modes of action for renal preservation. For example, it is an effective FRS for OH–,[15] a detoxifier,[16] and an indispensable constituent of intraoperative hydration protocols for the prevention of acute renal failure after renal cadaveric transplantation.[17] On the other hand, glucose, which is currently used for its hyperosmolar effect in Collins-2 flush solution, has been shown to exacerbate acute renal ischemic damage in dogs.[18]

Adenosine[19,20] and magnesium[21,22] were added to PB-2 solution because they have been shown to improve the postreperfusion microcirculation and to enhance adenine nucleotide generation. Magnesium also exhibits a vasodilator effect and acts as a metabolic inhibitor,[21] helping to conserve intracellular energy stores during cold storage.

The overall composition of PB-2 (*Table 1*) differs from the Collins-2, Belzer Perfusate, and UW-1 flush

solutions. Good results with UW-1 solution have recently been reported[23,24]; however, significant improvement in renal preservation has not been clinically demonstrated. A direct comparison of UW-1 with PB-2 has not been done.

Sacks-2 flush solution which, like PB-2, contains mannitol, has an intracellular ionic composition and concentration, and is hyperosmolar.[25] The hyperosmolarity of the Sacks-2 solution (410 mOsm/kg to 430 mOsm/kg) may greatly increase the tendency of mannitol and magnesium phosphate to precipitate out of the solution within the kidney during cold storage[21]; this would cause direct renal injury.

Compared with Collins-2 solution,[21,26] Sacks-2 solution shows inferior preservation and failure to adequately achieve 48 hours of safe preservation.[27] This inadequacy of preservation may be due to the lack of adenosine in Sacks-2 and to inferior buffering capacities as reflected by its greater acidity (pH, 7.0).

In formulating PB-2, adenosine was added and acidity was lowered (pH, 7.25). The salutary features of Sacks-2 solution are probably related to its "flush solution effect."

TABLE 1
COMPOSITION OF INTRACELLULAR RENAL FLUSH SOLUTIONS (g/L)

	PB-2 flush	Collins-2 flush	Sacks-2 flush	Belzer perfusate	UW-1 flush
KH_2PO_4	2.05	2.05	4.16	3.4	3.4
$K_2HPO_43H_2O$	9.70	9.70	9.70	—	—
KCl	1.12	1.12	—	—	—
$KHCO_3$	—	—	2.30	—	—
Mannitol	25.0	—	37.5	—	—
Glucose	—	25	—	1.5	—
$MgSO_47H_2O$	3.70	7.38	—	8	1.2
$MgCl_2$	—	—	(2 mEq/mL)	—	—
Adenosine	1.0	—	—	1.3	1.34
Sodium glutathione	—	—	—	17.5	0.92
Albumin	—	—	—	5.3	—
$NaHCO_3$	0.84	0.84	1.26	—	—
Allopurinol	—	—	—	0.113	0.113
K^+-Lactobionate	—	—	—	—	39.8
Raffinose	—	—	—	—	17.8
Hydroxyethyl starch	—	—	—	—	50
Osmolality (mOsm/kg)	340	320	430	300	320-330
pH	7.25	7.00	7.00	7.10	7.40

PRELIMINARY RESULTS WITH PB-2 SOLUTION

Ten canine renal autotransplants, preserved using PB-2 flush solution, were compared with 10 preserved using conventional Collins-2 solution. After 50 hours of cold storage, overall improvement in renal recovery and viability were measured by recipient post-transplant inulin clearance and survival. The measures were significantly greater with PB-2 than with Collins-2.

At 1 week post-transplant, mean serum creatinines were 2.88 mg/dL for the PB-2 group and 12.18 mg/dL for the Collins-2 group. This paralleled the post-transplant inulin clearance of 42.9 ± 33.8 mL/min for PB-2 *v* 14.6 ± 16.0 mL/min for Collins-2 ($P < 0.001$). Survival at 1 week post-transplant was also significantly greater ($P < 0.01$) for the PB-2 group (80%) than for the Collins-2 group (30%). In this study, characterization of PB-2 solution's cellular preservation mechanisms by HPLC, ^{31}P-MRS, and electron microscopy studies indicate that this solution enhances renal preservation both by diminution of reperfusion injury and by maintenance of intracellular high energy metabolites that are necessary for viability.[28]

These studies indicate that organ preservation can be improved if researchers are guided by an understanding of specific cellular interactions that occur before and after transplantation. Studies are necessary to verify that the results demonstrated in animals are applicable for clinical renal transplantation. Trials using PB-2 for human transplant preservation are being planned. Our findings support the hypothesis that a significant improvement in outcome of renal transplants can occur with the use of an improved cold storage solution.

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