Urological Research 1, 83-87 (1973)

In-vivo and Ex-vivo Renal Preservation
Preliminary Observations

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Summary. A new renal perfusate of modified intracellular electrolyte composition made hyperosmolar with mannitol and requiring no additional additives was successfully used to preserve canine kidneys "ex-vivo" for 48 hours by initial perfusion and hypothermic storage. - The new perfusate was also successful in protecting totally ischemic canine kidneys from the lethal effect of two-hours of normothermic exposure "in-vivo". - The preservation technique is uncomplicated, the materials involved are inexpensive, and the preservation apparatus is readily transportable.

Key words: Renal preservation, renal transplantation, hypothermia, normothermia, hyperosmolar intracellular electrolyte solution.

Introduction

The increasing use of cadaver kidneys for human allotransplantation has stimulated the search for a simple, effective means of extracorporeal renal preservation. One or two day ex-vivo renal preservation would grant time to establish the most ideal donor-recipient histocompatibility and would permit long distance transportation of donor kidneys. The increasing employment of renal autotransplantation had necessitated investigation with regard to methods of protecting the in-vivo ischemic kidney from the effects of prolonged normothermic exposure that do not require continuous cooling. Accordingly, we have sought to discover a simple inexpensive method of renal preservation applicable to both short-term normothermic ischemia, and long-term hypothermic ischemia.

Success in both areas has been experimentally achieved in our laboratory by utilizing a new renal perfusate of modified intracellular electrolyte composition rendered hyperosmolar with mannitol. Specifically, four canine kidneys were successfully preserved for 48 hours after initial perfusion with this solution followed by hypothermic storage and reimplantation. In addition, four canine kidneys were completely protected from the otherwise irreversibly damaging effects of two hours of normothermic ischemia simply by initial perfusion with this solution followed by contralateral nephrectomy, and autotransplantation.

Materials and Methods

Mongrel dogs of both sexes weighing between 15 and 30 kilograms were used. In the 48 hour renal preservation studies, right nephrectomy has been performed at least four weeks prior to removal and storage of the remaining kidney. Thus, these dogs were rendered anephric during the two day renal preservation period. In the two hour normothermic ischemia experiments, contralateral nephrectomy was performed just prior to autotransplantation of the preserved kidney. Postoperative renal function in both experimental models was totally dependent upon the preserved kidney.

* Supported by the Max Kade Foundation, New York, New York.

Food and water were withheld during the night prior to operation. Anesthesia was induced and maintained with sodium pentobarbital and hydration was maintained with 1500 ml. of five per cent dextrose in 0.45 per cent saline administered intravenously throughout the operative period. Intermittent positive pressure ventilation with 40 per cent oxygen was used, and all operations were performed through an abdominal midline approach. Mannitol (12.5 gms) was given by rapid intravenous administration five minutes prior to interrupting the renal circulation and again just after the circulation had been reestablished. The profound fall in intrarenal vascular resistance caused by the intravenous mannitol (8) allowed excellent flow of the perfusate and obviated the need to include vasodilators. The warm ischemia time (from interruption of the circulation to perfusion of the kidney) for the 48 hour experiments averaged three minutes.

Following its removal, a kidney to be preserved for 48 hours was placed in 200 ml of precooled perfusion (2° C) solution in a sterile basin surrounded by ice. Cooled perfusate (6° C) was then allowed to flow through the kidney by gravity from a height of 100 centimeters (73.5 mm. Hg.). Two hundred ml. of precooled perfusate were used and the perfusion period averaged five minutes. Following this brief perfusion the kidney was immersed for 48 hours in 1 000 ml. of the hyperosmolar intracellular electrolyte solution which was maintained at 2° C by means of a thermostatically controlled cold probe (Forma Scientific Inc., Marietta, Ohio). Subsequent to its removal from the storage canister, the kidney was reimplanted into the dog's left iliac fossa. Vascular anastomoses were completed in approximately 30 minutes, and a tunneled ureteroneocystostomy was performed.

The two hour normothermic ischemia experiments were performed in a similar manner except that the kidneys were briefly perfused i in-vivo and without further cooling wer simply replaced in the renal fossa for the stated period until the circulation was reestablished by autotransplantation. The ureters were left intact in these experiments, and blood flow to the kidney via the ureter was prevented by a "bulldog" clamp placed on the ureter during the period of ischemia.

Renal function was assessed by serum creatinine determinations and the dogs were considered to have resumed "normal" renal function when postoperative serum creatinines returned to individual prenephrectomy levels.

The hyperosmolar intracellular electrolyte solution described herein (Table 1) is of the electrolyte composition created by Collins (4) with the notable exceptions that it contained none of the additives he advocated (dextrose, phenoxybenzamine, procaine, heparin), and contained no magnesium sulfate. Moreover, this basic "intracellular" electrolyte solution (osmolarity 190 mOsm/L without additives) was rendered hyperosmolar (410 mOsm/L) upon the addition of 50 grams of mannitol.

Table 1. Characteristics of perfusate and storage solution

Potassium	115 meq/liter
Phosphate	110 meq/liter
Sodium	10 meq/liter
Chloride	15 meq/liter
Bicarbonate	10 meq/liter
Mannitol	50 grams/liter
pH	7.3 at 2° C
Osmolarity	410 mOsm/liter

Results

Serum creatinine values in the four consecutive dogs rendered anephric by removal and 48 hour storage of their remaining kidneys rose predictably during the preservation period from normal levels to an average of 8.3 mg%. Following reimplantation of the stored kidneys urine production was immediate and serum creatinine values fell dramatically, in a reproducible manner, to prenephrectomy levels within six days (Tables 2 and 3).

Serum creatinine levels in the four consecutive dogs that underwent autotransplantation after two hours or normothermic ischemia demonstrated a transient rise averaging 0.6 mg% during the first one or two postoperative days followed by a rapid return to preoperative levels within five days (Table 4). The transient rise in serum creatinine in these experiments is slightly greater than might be expected from the loss of the contralateral kidney.

Discussion

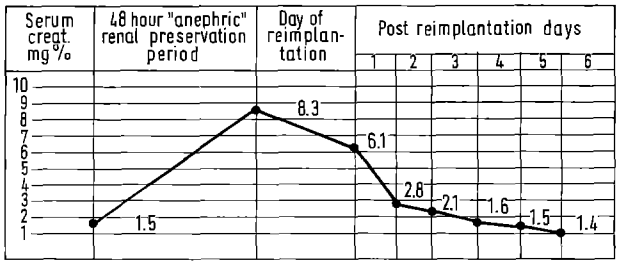
Preservation of the canine kidney by hypothermia alone is limited to 12 hours (5). Collins (4) extended this period of preservation to 30 hours in 3 dogs by initially perfusing these kidneys with an "intracellular" electrolyte

Table 2. Serum creatinine determinations: 48 hour renal preservation experiments - anephric dogs

	48 hour "anephric" renal preservation period	Day of Reimplantation	Post reimplantation days							
			1	2	3	4	5	6	9	10
DOG 19	1.3	7.3	6.1	3.4	2.5		1.7	1.3		
DOG 20	1.2	8.2	5.2	2.2	1.8		1.3			1.1
DOG 21	1.8	8.9	5.8	2.9	2.1		1.8		1.7	
DOG 22	1.5	8.9	7.4	3.0	2.0	1.5	1.5			1.9

Serum creatinine: mg.%

Table 3. Average serum creatinine following renal reimplantation into anephric dogs after 48 hour storage



solution (Table 1) to which he added magnesium sulfate, dextrose, procaine HCl, sodium heparin and phenoxybenzamine. We have accomplished 48 hour preservation of 4 consecutive canine kidneys with a perfusate of the same electrolyte composition described by Collins (4). (less magnesium sulfate), but with the notable exceptions that is contained none of the additives he advocated, and that it was made hyperosmolar with the addition of mannitol.

The experimental model we designed necessitated immediate and sustained function of the preserved kidney. Renal function upon reimplantation was indeed immediate and the high serum creatinine levels (8.3 mg%) created by the two day anephric interval fell rapidly to prenephrectomy levels (1.5 mg%) by the 6th post-reimplantation day. In contrast, the return to normal function (BUN less than 30 mg%) in the five successful 24 hour canine renal preservations reported by Belzer (2) occurred by 12 days. Therefore, renal function returned twice as quickly in the dogs whose kidneys we preserved for 48 hours by initial perfusion and hypothermic storage than in the dogs whose kidneys were preserved by Belzer for 24 hours by continuous pulsatile perfusion.

Moreover, we know of no methods that afford complete protection of the ischemic in-vivo canine kidney than do not require continuous cooling. The fact that simple, initial perfusion of the ischemic canine kid-

Table 4. Serum creatinine determinations: 2 hour normothermic renal ischemia. experiments

	Day of auto-transplantation	Post Autotransplantation Days					
		1	2	3	4	5	10
DOG 24	1.2	1.7	1.6	1.3	1.1		1.2
DOG 26	1.7	2.3	2.8	1.8	1.8		1.7
DOG 28	1.1	1.7	1.9	1.5	1.3	1.1	1.2
DOG 32	1.4	2.1	1.9	1.7	1.5	1.3	

Serum creatinine: mg%

ney with the cold hyperosmolar "intracellular" electrolyte solution we have described can protect it from the lethal effects of two hours of normothermic exposure (3) attests to the efficacy of this solution and suggests numerous applications for its use.

Theoretical Considerations

The normally respiring renal cell maintains a distinct intracellular electrolyte composition high in potassium, magnesium, and phospate, low in sodium chloride, and slightly electronegative by virtue of the energy requiring sodium "pump" (6, 12). Deprived of circulation, hence, energy, the sodium pump ceases to function and sodium and chloride readily traverse the permeable cell membrane into the cell yielding to both chemical and electrical gradients (10). Potassium and to a lesser extent magnesium are conversely rapidly lost from the cell interior (9) (phosphate is variably bound to intracellular proteins). The result of these rapid changes in ion distribution in the ischemic cell is a net gain, not merely an exchange, of intracellular ions (sodium and chloride) followed passively by water (11). and a profound loss of potassium and to a lesser extent magnesium (9). Moreover, the remaining non-diffusible intracellular molecules (with electronegative charge) exert Donnan, and osmotic forces resulting in the further movement of water into the cell (11). The morphologic consequence of these changes in ion and water distribution is characterized pathologically as "cloudy swelling", and "hydropic degeneration". The physiologic consequence of this cellular swelling includes the physical phenomenon of "failed reflow" of blood through the kidney (14, 15) associated with marked elevation of intrarenal resistance often erroneously attributed to "vasospasm". Red blood cells have, in fact, been shown to impact in the swollen glomerular capillary bed of kidneys subjected to ischemia after the macrocirculation has been reestablished (15). Efferent flow to the peritubular capillaries is thereby markedly reduced. The ischemic insult to the renal tubular cell may thus be perpetuated even after the macrocirculation has been reestablished, as seen in the familiar transplant kidney of dusky color and poor flow.

It would appear logical therefore, to attempt to prevent these changes in ion and water distribution by providing the ischemic renal cell with an extracellular environment approximating the intracellular environment. A renal perfusate similar in electrolyte composition to the cell interior would discourage changes in ion distribution on both sides of the permeable cell membrane, i.e., potassium and magnesium loss, and sodium and chloride (and water) gain by the cell. Keeler (9) and Collins (4) have demonstrated this convincingly. In addition, however, the osmolarity of the extracellular environment is critical in preventmacrocirculation. This has been impressively demonstrated by Flores and Leaf (7). Mannitol was chosen as thepotentosmotic agent because it generally remains extracellular, has little known toxicity to the kidney, and because it is not metabolized. Previous investigation in this laboratory has demonstrated the protective effect of systemically administered mannitol upon the ischemic kidney (13). Mannitol as we are using it probably exerts its effect by countering the Donnan, and osmotic influence of the negatively charged non-diffusible intracellular protein molecules, thereby not only preventing movement of water into the cells, but also by slightly dehydrating the cells which subsequently allows for increased blood flow through the kidney upon reestablishment of the circulation (7).

The independently protective effects of intracellular electrolyte solutions and hyperosmolar solutions are at least additive, if not synergistic when used in combination. Histological evidence in support of this concept has recently been presented by Acquatella (1). He has clearly demonstrated that perfusion of the ischemic kidney with Ringer's lactate solution results in "immediate" cellular swelling associated with marked sodium and chloride gain by the cell, and profound potassium loss. Perfusion with hyperosmolar Ringer's lactate (200 mm glucose added) helped to prevent the cellular swelling, but did not prevent the changes in electrolyte distribution as previously described. Conversely, perfusion with Collins' C_2 solution prevented the changes in electrolyte distribution, but cellular swelling persisted. Both of these phenomena could be prevented, however, when the ischemic kidney was perfused with a hyperosmolar intracellular electrolyte solution (Collins' C_2 solution + 60 mM K_2SO_4 + 20 mM $NaHO_3$ + 200 mM glucose). This solution (which was not actually tested) is conceptually similar to the one which we have advocated with the notable exceptions that we have eliminated the "SO_4" anion entirely because it is not a usual intracellular component, have added mannitol as the osmotic agent for the reasons previously mentioned, and have eliminated all unnecessary additives.

Conclusions

Collins (4) undoubtedly foresaw improvements in his C_4 solution when he stated that "there is no reason to presume that the concentration of ions in the new perfusate is the

best that can be achieved for dogs or man. The good results, especially after the modifications introduced, provide a starting point for further improvements."

We feel that significant improvements in the field of hypothermic and normothermic renal preservation have been made by combining the independently protective effects of "intracellular" electrolyte solutions and hyperosmolar solutions, and by eliminating apparently unnecessary additives. We are encouraged by the preliminary results reported herein and plan further experiments to validate and extend existing preservation periods.

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