organ preservation:

Hypothermic Renal Storage.
II. Effect of Vasoactive Drugs with Diverse Mechanisms of Action

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INTRODUCTION

The two most commonly used techniques for long-term preservation of donor kidneys prior to transplantation are cold perfusion with an intracellular-like electrolyte solution followed by static hypothermic storage and pulsatile hypothermic perfusion developed by Belzer.[1] The latter method has two distinct advantages. First, longer preservation with resultant good renal function is possible. Second, viability of the kidney can be assessed pre-operatively. Cold perfusion or "washout", however, is simple, inexpensive and easily implemented at hospitals peripheral to the transplant center. Ideally, cold storage must afford preservation of sufficient duration to guarantee ultimate function of the transplant following both transport to a nearby transplant center and transplantation.

Collins showed in 1969[2] that a "washout" solution which was similar in composition to intracellular fluid was capable of preserving kidneys for 30 hours. Since then, his work has been extended by several investigators[3,4,5,6,7], and although the specific compositions of the various solutions differ slightly, it seems clear that an intra-cellular type fluid is superior for static hypothermic storage. Collins' original solution (C4) contained phenoxybenzamine which was said to act as an alpha blocker and as a stabilizer of lysosomal membranes.[2] It was subsequently reported by Terasaki,[8] however, that the phenoxybenzamine had lost most of its activity under these circumstances of cold storage. Whether a vasoactive drug included in the "washout" solution is beneficial at all has not yet been clearly established. It is the purpose of the present study to determine the effects of several vasoactive drugs with diverse mechanisms of action in combination with Collins' intracellular solution.

MATERIALS AND METHODS

Groups Studied

Twenty female mongrel dogs were randomly allocated to four groups as follows:

Group I: Five animals' kidneys were perfused with Collins' C3 solution. This solution contained no vasoactive drug. The composition is shown in Table I.

Group II: Five animals' kidneys were perfused with Collins' C4 solution. Phenoxybenzamine was added to room temperature solution 24 hours prior to the study. Clearing of turbidity usually occurred within 2 hours and the solution was cooled to 0-4°C.

Group III: The Collins' C3 was used in five animals as the intial "washout". Papaverine HCl, 0.060 gm/L, was added to the solution immediately prior to use.

Group IV: Collins' C3 solution with chlorpromazine, 0.050 gm/L, was used to perfuse five other animals' kidneys.

Procedure

Each animal underwent left nephrectomy under fluothane (Halothane)* anesthesia. The kidney was immersed in a saline bath at 0-4°C, and intra-arterial "washout" was

*Kindly supplied by Ayerst Laboratories

TABLE I: COMPOSITION OF WASHOUT SOLUTION FOR GROUP I.

KH_2PO_4	15	mEq/L
$K_2HPO_4 \cdot 3H_2O$	85	mEq/L
KCl	15	mEq/L
$NaHCO_3$	10	mEq/L
$MgSO_4 \cdot 7H_2O$	60	mEq/L
Heparin	5000	u/L
Procaine HCl	100	mg/L
Glucose	25.5	gm/L

performed with one of the above four solutions for five minutes. The kidney was then stored at 0-4°C for 24 hours. Following the storage period, the kidney was transplanted into the contralateral iliac fossa of the same animal. Arterial and venous anastomoses were accomplished in an end-to-end fashion to the iliac vessels using Nakayama staples. Ureteroneocystostomy was carried out through a submucosal tunnel. The animal's other kidney was removed. Each animal received 500 cc normal saline plus 12.5 gm mannitol during the nephrectomy, and 1000 cc normal saline plus 25.0 gm mannitol during the transplantation procedure.

Serum creatinine levels were obtained every one to two days for the first 14 days, then weekly. Clearances of inulin and PAH were measured in the single contralateral control kidney prior to transplant and in the preserved kidney immediately following removal of the control kidney. In animals which survived, clearances were obtained at weekly intervals.

Results

All animals survived the operative procedures. In Group I, there were two post-operative deaths. One animal died at five days of undetermined cause, another at 11 days of pneumonia. Both had serum creatinine levels of 1.8 mg% at the time of death, and their maximum postoperative creatinines were 2.7 mg% and 2.6 mg% respectively, so uremia probably had not contributed to their death.

In Group II, two animals died within the first postoperative week. Serum creatinines at the time of death were 5.5 mg% and 8.9 mg%, and the deaths were attributed to renal failure. One animal was noted to have a large renal infarct at autopsy which undoubtedly was due to a technical problem. The other animal had no gross abnormality of the kidney.

In Group III, all animals had died by the end of the first post-transplant week. All were severely azotemic and death was therefore attributed to renal failure.

In Group IV, three animals died in the early postoperative period. One was found to have an intraperitoneal hemorrhage, the source of which could not be found. The serum creatinine in this animal was 13.8 mg% suggesting the possibility of uremia as a contributing cause of the hemorrhage. In the other two, the serum creatinines were 6.5 mg% and 15.5 mg%, and death was clearly attributable to renal failure. Mean serial serum creatines $\pm$ S.E.M. for the four groups are shown in Figure 1.

Table II summarizes survival time and creatinine level at death in the four groups. These data were evaluated by Tukey's comparison following analysis of variance (Table III). The results in Group III (papaverine) were significantly worse (p value < 0.05) than Group I (controls). No other comparisons were statistically significant although the difference between Group IV and Group I approached statistical significance.

Inulin and PAH clearances were significantly (p value < 0.05) depressed in all kidneys immediately post-transplant. In the animals that survived beyond the first week, clearances returned to normal with no differences between the groups.

Discussion

The mechanism of action of intracellular type fluid in kidney preservation has not been precisely determined. Some investigators[5,9,10] have felt that it acts by preventing the depletion during storage of important intracellular ions such as potassium and magnesium. Collins[11] suggested that the efficacy of an intracellular solution was in a large part dependent on the magnesium stability. Collste[3] believes that although magnesium stability is important, potassium stability is of equal importance. Watkins, et al.[7] reported that magnesium was completely unnecessary. Despite this lack of concensus, however, there is general agreement that a perfusion solution which is hyperosmolar and of similar

TABLE II: ISCHEMIA TIMES AND RESULTS IN GROUPS I-IV

Group	No.	Minutes of warm ischemia	Anastomosis time in minutes	Total Ischemia time	Perfusion Solution	Vaso-active drug	Survival (days)	Cause of death	Creat. at death (mg%)
	46	2	9	24:25			11	pneumonia	1.8
	84	2	6	26:32			112		1.6
I	61	2	4	24:35	C3	None	31		1.8
	81	2	6	24:33			5	undetermined	1.8
	17	2	7	24:40					2.0
	89	3	7	24:30			7	uremia	5.5
	44	2	8	25:20			29		1.6
II	70	2	8	25:51	C4	PBA	29		1.2
	8	1	7	25:12			5	uremia/renal infarct	8.9
	53	2	5	24:23			84		1.5
	103	2	6	23:39			3	uremia/peritonitis	5.7
	42	1	5	24:22			3	uremia	6.1
III	83	1	6	25:25	C3	PPV	7	uremia	18.6
	82	2	6	24:18			2	uremia	4.0
	31	1	6	24:04			5	uremia	9.7
	281	2	6	24:02			112		1.3
	65	1	5	24:30			3	undetermined	6.5
IV	5	2	7	23:50	C3	CPZ	10	uremia	15.5
	42	1	6	24:16			7	intraperit. hemorrhage	13.8
	20	1	6	24:40			29		2.0

ionic composition to intracellular fluid is superior to extracellular-like fluid for long-term kidney preservation. Moreover, there is some evidence[4,9,12,13] that even in systems utilizing continuous perfusion, a perfusate similar to intracellular fluid is of benefit.

The mechanism of action of the drugs added to the solution is not clear. In Collins' original description of C4 solution,[2] he stated that phenoxybenzamine (PBA) was added to produce alpha adrenergic blockade and lysosomal membrane stabilization. Subsequent evidence,[8] however, has shown that PBA deteriorates rapidly in solution and may be inactive when used as originally described. Woods[14] used a lower dose of PBA than originally described with no deleterious effect. Watkins[7] actually showed slightly improved results when PBA was not added to the perfusion solution. Several investigators[13,15,16,17] have reported improved post-transplant function following direct intra-arterial injection of PBA. Braf[15] compared directly Collins' solution with and without PBA and concluded that it was not beneficial in the absence of prolonged warm ischemia. The consistent warm ischemia time of under two minutes in this study may explain the lack of benefit of PBA.

Chlorpromazine (CPZ) is both an alpha adrenergic and a lysosomal membrane stabilizer.[18] In the present study, CPZ was added to the preservation solution immediately prior to use, so there can be little doubt as to its activity. It clearly exerted no beneficial effect and in fact, seemed to yield poorer preservation although results were not significantly different from controls.

Papaverine is a nonspecific vasodilator and has been used to overcome vasospasm in kidneys prior to transplantation. The addition of this drug to cold preservation solution, however, clearly exerted a deleterious effect. The mechanism by which this occurs is not clear. It may be as Braf[15] found with PBA, that vasoactive drugs are useful only when there is a significant degree of warm ischemia and vasospasm.

Under the conditions of this study, however, which included a minimal warm ischemia time and active mannitol diuresis, vasoactive agents seem to be of no benefit and may be harmful. Since these conditions are analogous to the clinical situation of the living related donor, and many of the cerebral death-beating heart cadaver donors, the addition of vasoactive drugs to the "washout" solutions in these situations should be approached with caution.

TABLE III: ANOVA Summary Table

SOURCE	DF	SS	MS	F
Between	3	165.9215	55.307	2.489
Within	16	355.5080	22.219	
Total	19	521.4295		

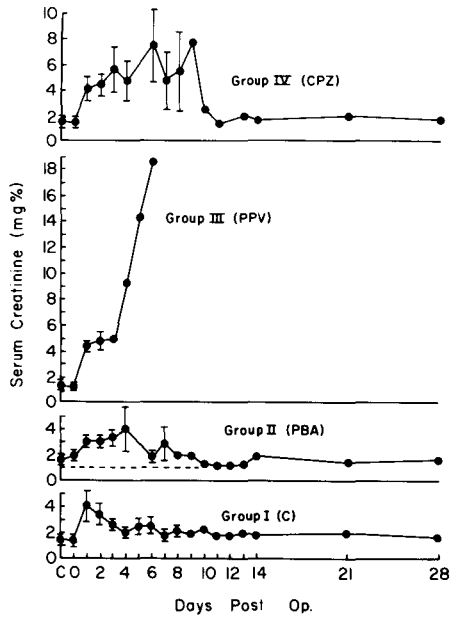

Fig. 1. Comparison of mean serum creatinine levels with ± S.E.M. in all groups studied.

SUMMARY AND CONCLUSIONS

The use of vasoactive drugs as a component of the "washout" solution in hypothermic renal storage was studied. Twenty dogs underwent nephrectomy. The kidneys were stored for 24 hours following "washout" with Collins' C3 (5 dogs); Collins C4 (5 dogs); C3 plus papaverine (5 dogs); and C3 plus chlorpromazine (5 dogs). All animals then underwent autotransplantation and immediate host nepherctomy. No group was significantly better than Control (Group I). Group III (papaverine) results were significantly worse. All animals in Group III died of renal failure within the first postoperative week. No other differences were significant.

It is concluded that: (1) in the absence of significant warm ischemia, "active" vasoactive drugs are of no benefit in hypothermic renal preservation; (2) papaverine in the "washout" solution is harmful and should not be used; and (3) the addition of any vasoactive drug to the cold washout solution should be undertaken with caution.

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