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The Effect of Two Different Renal Preservation Methods on Canine Renal Allograft Survival

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Summary. Preservation of human cadaver kidneys for transplantation has been achieved primarily by two methods, hypothermic pulsatile perfusion with cryoprecipitated plasma and cold storage with an electrolyte solution. It has been suggested that pulsatile perfusion results in an increased antigenicity of the transplanted kidney. To investigate the possibility that pulsatile perfusion causes changes which may accelerate allograft rejection, machine preservation was compared with simple cold storage. The kidneys were preserved by either one of the two methods for 6 or 24 hours followed by allotransplantation in nephrectomised dogs. No immunosuppressive drugs were given.

Kidneys which were allografted without undergoing any preservation (0 hrs) had a mean survival time of 10.4 ± 1.7 days (n = 5). Kidneys preserved by machine perfusion for 6 and 24 hours survived for 9.6 ± 1.4 (n = 5) and 10.9 ± 1.3 (n = 9) days respectively. The mean survival time for simple cold storage for 6 and 24 hours was 9.3 ± 1.3 (n = 7) and 12.0 ± 1.9 (n = 6) days. Our findings suggest that in kidneys exposed to minimal warm ischaemia there is no significant difference between the two methods of preservation on renal allograft survival for the time intervals tested.

Key words: Kidney preservation - Cold storage - Perfusion - Allografts - Survival.

Preservation of human kidneys for transplantation has been achieved mainly by either hypothermic pulsatile perfusion or cold storage with an electrolyte solution. The merits of the two methods have been compared clinically and experimentally in many reports. Clark et al. (4) reported that cadaver kidneys preserved by flushing with Collins solution and simple cold storage functioned better after transplantation than kidneys preserved by hypothermic pulsatile perfusion. This study raised the possibility that pulsatile perfused kidneys are rejected more frequently than cold stored kidneys. Although present reports from various transplant centres show essentially no difference in the rate of rejection between perfused and non-perfused kidneys, a true evaluation of a comparison between the two methods of preservation is difficult because of the many uncontrolled variables (6, 7, 11).

Most experimental studies comparing the two preservation methods have been done by evaluating renal function after reimplantation of the autografted kidney following a variable period of preservation. We have compared the two methods using the allografted canine kidney model. Such an approach would demonstrate the additional rejection factor which is not present in the autograft studies. The relative merits of the two methods were assessed by the survival time of the allografted kidney.

METHODS

Thirty-two female mongrel dogs, subdivided into five experimental groups, were used. The

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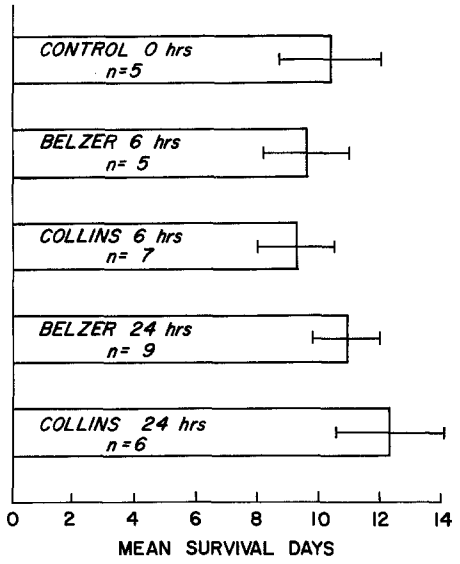

Fig. 1. Comparison of survival times in dogs in which allografted kidneys were preserved by two different methods for 6 and 24 hours. Values represent mean survival times in days plus the standard error of the mean

animals were anaesthetised with sodium thiamylol (Surital, 15 mg/kg, i.v.) and maintained with 2-Bromo-2-chloro 1,1,1, trifluoroethane (Halothane) on a Mark 7 respirator (Bird). The animals were deprived of food and given free access to water during the 24 hours prior to the experiment. Five different experimental protocols were followed. In the first group (control, n = 5) the kidney was removed through a midline abdominal incision after the animal had received 500 ml of a 5% dextrose in 0.45% saline solution containing 1.2% mannitol intravenously. The renal artery was cannulated and the kidney flushed with 200 ml cold (4° C) Ringer's lactate solution. The kidney was then immediately transplanted into another recipient dog which had been prepared (bilateral nephrectomy) to receive the kidney.

In all the groups, the kidneys were allografted in the following manner. The animals were prepared in the same fashion as described and both kidneys removed. The donor kidney was reimplanted into the iliac fossa by an end-to-end anastomosis between the renal artery and the iliac artery and by an end-to-side anastomosis between the renal vein and the iliac vein. The ureter was reimplanted into the urinary bladder through a sub-mucosal tunnel. During the reimplantation, the recipient animal received 500 ml i.v. of a 5% dextrose in 0.45% saline solution containing 1.2% mannitol.

In groups two and three, the kidneys were subjected to either 6 hours of machine preservation (n = 5) or 6 hours of cold storage (n = 7) respectively. For these 6 hour comparative studies, both kidneys from the same animal were removed providing each kidney had a single renal artery. The renal artery of each kidney was cannulated. One kidney was flushed with 200 ml (4° C) Collins C_2 preservation solution and subsequently stored under sterile conditions in Collins C_2 ice-slush solution (4° C). The other kidney was flushed with 200 ml cold (4° C) Ringer's lactate solution and then placed on a Belzer preservation unit (LI-400) where it was maintained by hypothermic (6-9° C) pulsatile perfusion (60 mm Hg maximum systolic pressure) with cryoprecipitated dog plasma. During the perfusion period, perfusate pH, temperature and pO_2 and pCO_2 were monitored (IL 230 blood gas analyser). After six hours of preservation by either method, the kidneys were allotransplanted.

In the final two groups (four and five), the kidneys were subjected to either 24 hours of machine preservation (n = 9) or 24 hours of cold storage (n = 6) respectively. The procedures were identical to those just described with the exception that the kidneys were preserved for a longer period of time.

In all cases, post-operative care consisted of two days of intravenous feeding (500 ml/day, 5% dextrose in 0.2% saline). Food and water were also withheld for 24 hours after surgery. For prophylactic reasons, antibiotic therapy consisted of 1.2×10^6 units Bicillin i.m. and 6×10^5 units Wycillin i.m. on the day of nephrectomy. Wycillin alone was continued for the next five days. No immunosuppressive drugs were given. Peripheral venous blood samples were taken at various intervals until death to monitor serum creatinine. Cortical biopsies for light microscopy were taken at the time of nephrectomy, and upon restoration of blood flow to the kidney and at the time of death. An autopsy was done on all the dogs to ascertain the cause of death.

The Collins C_2 solution used was made up as described by Collins et al. (5). The plasma used for hypothermic pulsatile perfusion was obtained commercially (Pel-Freez Biological Inc.). The plasma was pooled in 10 litre batches for the experiments. The plasma was cryoprecipitated and filtered through a series of millipore filters to remove labile lipoproteins. The following drugs were added to 750 ml of plasma: 8.12 mEq magnesium (as Mg_2SO_4), 40 units insulin (Iletin, R-40), 10^6 units potassium penicillin G, and 125 mg methyl-prednisolone sodium succinate (Solu-Medrol, Upjohn).

Table 1. Number of surviving animals following renal allotransplantation

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Control (5) 0 hrs	5	5	5	5	5	5	5	5	3	2	1	1	1	1	1	1	1	
Belzer (5) 6 hrs	5	5	5	5	5	5	4	3	3	3	2	1	1	1				
Collins (6) 6 hrs	6	6	6	6	6	6	5	5	5	4	2	1	1	1	1			
Belzer (9) 24 hrs	9	9	9	9	9	9	8	8	7	4	4	4	3	2	1	1	1	1
Collins (6) 24 hrs	6	6	6	6	6	5	5	5	5	5	4	4	3	3	3	3		

Control (0 hrs) corresponds to immediate renal allotransplantation.
Numbers represent living animals at each day.

Table 2. Serum creatinine levels (mg%) following renal allotranplantation

Day	0	1	2	3	4	5	6	8	10	12	14	16	18
Control	0.8	2.7	3.9	4.6	4.4	4.9	5.0	8.1	10.2	10.7	12.0	11.7	9.2
0 hrs	0.1	0.4	0.9	1.0	1.0	1.2	1.5	2.1	1.6				
Belzer	1.2	3.2	3.3	3.9	4.5	6.0	8.0	15.0	19.3				
6 hrs	0.1	0.6	0.9	1.8	2.3	2.4	2.6	3.7	4.6				
Collins	1.2	2.8	3.7	5.5	6.6	6.7	6.6	8.0	12.4				
6 hrs	0.1	0.4	0.8	2.1	2.8	4.4	3.3	1.5	2.3				
Belzer	1.0	4.5	5.3	5.9	7.4	9.0	8.1	11.8	10.9	12.8	12.5	16.8	23.7
24 hrs	0.1	0.4	0.8	1.1	1.7	2.6	1.8	2.1	3.1	2.7	2.0		
Collins	1.1	5.1	7.5	8.0	5.8	6.0	5.9	8.3	12.2	17.9	20.9	26.5	
24 hrs	0.1	0.3	0.5	1.0	0.8	1.0	1.3	1.5	1.9	1.5	10.9	4.0	

Values represent means ± S.E.M.

Data were analysed by Kruskal-Wallis statistic (6). The 0.05 level of probability was used as the criterion of significance.

RESULTS

The survival times of the dogs in which kidneys preserved by the different preservation techniques were allotransplanted are presented in Figure 1. There was no statistically significant difference between the control group which survived for 10.4 ± 1.7 days and the four experimental groups. The dogs in which the kidneys preserved by the Belzer 6 hr and by the Collins 6 hr technique survived for 9.6 ± 1.4 and 9.3 ± 1.3 days respectively. For preservation by the Belzer 24 hr and by the Collins 24 hr technique, the corresponding survival times were 10.9 ± 1.3

and 12 ± 1.9 days. The daily survival rate of the animals in each group is listed in Table 1. All of the animals in each group were alive five days after allotransplantation but died at various times thereafter (range: 6 to 18 days).

Mean serum creatinine levels following renal allotransplantation are presented in Table 2. At day 1 the dogs in which the kidneys were preserved by the Belzer and Collins techniques for 6 hrs had mean serum creatinine levels which were not statistically different from the control group's value (2.7 ± 0.4 mg %). The mean serum creatinine levels of the Collins 24 hr group (5.1 ± 0.3 mg %) and the Belzer 24 hr group (4.5 ± 0.4 mg %) were statistically higher ($p = 0.01$) than the control group (2.7 ± 0.4 mg %) and the Collins 6 hr group (2.8 ± 0.4 mg %). Although the mean serum creatinine levels of the Collins 24 hr group (5.1 ± 0.3 mg %) were greater than that of the Belzer 24 hr group (4.5 ± 0.4 mg %) this difference was not significant.

At day 2 there was no statistical difference between the mean serum creatinine levels of the control group (3.9 ± 0.9 mg %) and the Belzer 6 hr group (3.3 ± 0.9 mg %) and the Collins 6 hr group (3.7 ± 0.8 mg %). The mean serum creatinine levels of the Collins 24 hr group (7.5 ± 0.5 mg %) were statistically greater than the control group (3.9 ± 0.9, $p = 0.04$) the Belzer 6 hr group (3.3 ± 0.9, $p = 0.004$) and the Collins 6 hr group (3.7 ± 0.8, $p = 0.02$). At day 3 the Collins 24 hr group had a statistically higher ($p = 0.05$) mean serum creatinine level (8.0 ± 1.0 mg %) than the control group (4.6 ± 1.0 mg %). Of special note is the fact that after day 3 no significant differences were detected between any of the groups. Serum creatinine levels progressively increased until the time of death.

Histological examination of the kidneys at the time of death showed evidence of severe rejection. This was characterised by mononuclear cell infiltration in the interstitium. None of the kidneys showed evidence of arterial or venous thrombosis or ureteric obstruction. Other reasons for cause of death were excluded by gross examination of the internal organs at the time of autopsy.

DISCUSSION

The limited availability of human cadaver kidneys calls for the adoption of the best techniques for the harvesting, preservation and transplantation of kidneys in order to achieve optimum results following transplantation. Most transplant centres in the U. S. A. have used machine perfusion preservations since Belzer and co-workers introduced their method for clinical purposes (2). However, when Collins et al. (5) showed that dog kidneys could be preserved for up to 30 hours with a simple cold flushing and storage method, several centres adopted this storage technique for human cadaver kidneys.

A proper clinical evaluation of the two methods, machine perfusion versus cold storage is difficult to interpret because the validity of certain conclusions has been based upon pooled data from various centres, the use of different harvesting protocols, differences in ages of the donors and unequal preservation times. Experimentally most researchers have been able to preserve dog kidneys with cold storage using Collins solution for 24 h if no warm ischaemic insult occurred at the time of nephrectomy. On the other hand, machine preservation has given better results if the kidney has been exposed to ischaemia prior to preservation (8, 10). From the experimental standpoint, it appears that either method of preservation can be used for 24 hours if the kidney has not been subjected to warm ischaemia. However, most of these experimental comparative studies were performed under ideal conditions and the kidney reimplanted into the same donor at the end of the preservation period (8). Consequently, possible immunological problems associated with the mode of preservation were never investigated.

Several investigators have suggested that machine perfusion may cause immunological damage to kidneys. Specifically, Anderson et al. (1) reported that pulsatile perfusion damaged the vascular endothelium and speculated that this injury exposed more antigenic sites. It has also been reported that the use of a plasma perfusate which contains cytotoxic antibodies can cause immune injury to the kidney and thereby induce rejection (9). A more recent report based upon one hour biopsies also noted that machine perfusion with plasma or albumin may cause changes in the kidney which can lead to decreased graft survival (13). These studies have collectively raised the question of immunological damage during machine perfusion. In spite of such valid objections, most centres are still using machine perfusion preservation. Perhaps, one of the reasons for the continued use of machine preservation may be the inadequate clinical testing and experience with simple cold storage preservation for periods longer than 24 hours.

The allograft survival time in our control group ranged between 9 and 17 days. For the two different preservation methods used in

the present experiments, the survival times were within this range, and there was no significant statistical difference between the control group and the Belzer or Collins groups for either 6 or 24 h of preservation. Likewise, there were no statistically significant differences between any of the possible comparisons among the Belzer and Collins groups. Consequently, based upon kidney survival times, there is no justification from the present data to say that machine perfused kidneys are rejected more vigorously than cold stored kidneys. Furthermore, the length of preservation time by either method for either 6 or 24 hours did not affect survival time. Dog kidneys subjected to minimal warm ischaemia can be preserved by either method for at least 24 hours without causing vascular and/or immunological damage which accelerates rejection. Scott et al. (12) have reported no difference in survival rate of transplanted human cadaver kidneys whether preserved by ice storage or machine perfusion. In their study, the warm ischaemia times were similar but the cold ischaemia times were not. Our results obtained in dogs agree with their conclusions.

The effect of the two different preservation techniques on renal function was also assessed by measuring the serum creatinine levels and did not show any major differences in renal function as a consequence of one or the other preservation technique. During the first two days, the Collins and Belzer 24 hour groups had significantly higher serum creatinine levels than the control, Belzer 6 hour and Collins 6 hour groups. However, these differences in renal function did not affect survival time. Presumably, therefore, preservation by machine perfusion did not cause any more or any less damage to the kidney than did simple cold storage as judged not only by survival rate but also by serum creatinine levels.

In conclusion, simple cold storage and perfusion preservation are equally effective for 24 hours when applied to kidneys exposed to minimal ischaemia removed from well prepared and stable donors. No differences in survival times could be demonstrated for machine preserved dog kidneys versus simple cold storage. Based upon our laboratory findings we see no objection to the use of machine perfusion. Conversely, we also see no objection to the use of simple cold storage for 24 hours when human cadaver kidneys can be harvested under optimal conditions.

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