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Long-term function of canine autograft kidneys that had been preserved extracorporeally for twenty-four hours

Preservation with hypothermia and pulsatile perfusion of specially treated plasma

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SUCCESSFUL short-term preservation of canine kidneys has been reported by several authors[1-3] in recent years, with data relating to the immediate functional and pathologic status of the preserved kidneys. Bauditz and associates[4] from our laboratory reported the immediate results of 24-hour preservation of canine kidneys by means of hypothermic pulsatile perfusion of treated homologous plasma and a film oxygenator. Our report presents the results of functional and pathologic studies of kidneys of six mongrel dogs that were killed after surviving for from 306 to 376 days (an average of 338.5 days) each with a solitary kidney autograft previously maintained extracorporeally for 24 hours by that method of preservation.

Methods

A series of eight dogs comprised the long-term study. All dogs were housed on a farm and fed a diet consisting of meat and Ken-L-Ration dog food. The renal function of each dog was maintained by a solitary kidney. One kidney of each of six dogs had been extracorporeally preserved for 24 hours, then reimplanted as an autograft, with a contralateral nephrectomy performed at the same time. The other two dogs served as controls. In each

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Table 1.—Optimal extracorporeal perfusion conditions for canine kidneys

Factor	Range or unit
Pressure, mm Hg	20 to 30/4 to 6
Flow rate, ml/g/min	0.15 to 0.6
Temperature, C	7.5 to 10
PO_2, mm Hg at 37 C	210
pH, at 37 C	7.28 to 7.34

control animal, the solitary kidney was simply flushed with heparinized saline and autotransplanted to the iliac fossa, with an immediate contralateral nephrectomy performed at the same operation. When killed, the two control dogs had survived 420 days, and 398 days.

The perfusate used for preservation of the kidneys was prepared in the following manner. Pooled commercial dog plasma was kept frozen until time of use. It was then rapidly thawed in a warm-water bath, and filtered through a 0.22-mμ millipore filter. The plasma was treated by this method for two reasons. First, labile low-density β-lipoproteins in the plasma were deliberately denatured by the freeze-thawing (cryoprecipitation) process and were removed by the millipore filtration. It is known[5] that the lipoproteins in untreated plasma used in a perfusion circuit are affected by liquid:gas and liquid:glass interphases and become denatured, aggregate, and form lipid emboli in the organ being perfused. Such lipid aggregates block renal capillaries and cause rising renal perfusion pressures, reduced perfusate flow rates, swelling of tissues, and ultimate cell death. Secondly, by passing the plasma through the sterilizing filter, all bacterial contamination was eliminated. The principal perfusion conditions maintained during the preservation of the six test kidneys are shown in *Table 1.*

When this follow-up study was performed, the animals had fasted overnight and blood was drawn for determination of blood urea nitrogen, serum creatinine, and serum sodium, potassium, chloride, and carbon dioxide estimations. Sodium pentobarbital anesthesia was then administered intravenously to each animal and a catheter was passed into the bladder. A fresh urine sample was evaluated for specific gravity, pH, protein and glucose content. In three test dogs and one control dog, exogenous creatinine clearance rates were determined by means of intravenous infusion of exogenous creatinine at a constant rate to maintain the plasma level in a range of 7 to 10 mg per 100 ml. Femoral artery puncture was done with a Rochester needle and the blood pressure recorded on a Sanborn polygraph.

Results

All of the eight dogs were in excellent health at time of the follow-up study. Three of the test dogs had an average weight gain of 4.03 kg (range 1.9

to 6.6 kg). The other three test dogs showed an average weight loss of 0.9 kg (range 0.1 to 2.4 kg). One of the control dogs had gained 1.7 kg and the other 4.4 kg. The average urine specific gravity was 1.026 (range 1.010 to 1.050) for the test dogs, and 1.022 for the control animals. The urine pH ranged from 6.0 to 8.0 in the test dogs and from 6.0 to 6.5 in the controls. No glycosuria was detected in any of the dogs. Two test dogs had a trace of albumin in the urine. The catheterized urine culture was sterile in all but one female dog in which it was difficult to perform the catheterization. The urine culture of that animal revealed 1,000 colonies of coagulase-negative *Micrococcus pyogenes var. albus* per milliliter of urine.

After reimplantation of the kidneys, initial increases in the blood urea nitrogen were minimal both in control and in test animals, and attained normal values in from three to five days after autotransplantation.

At the time of follow-up study, the average blood urea nitrogen for all the animals was 16 mg per 100 ml, ranging from 14 to 21 mg per 100 ml for the test dogs, and from 13 to 19 mg per 100 ml for the control dogs. The serum creatinine averaged 1.2 mg per 100 ml (range 0.9 to 1.4 mg per 100 ml) for five of the test dogs, and 1.3 mg per 100 ml for one of the control dogs. The serum electrolytes were normal for all the animals.

The exogenous creatinine clearance expressed in milliliters per kilogram of body weight per minute was 3.5, 3.7, and 2.4 ml for the three test dogs, and 3.36 ml for one of the control dogs. The mean arterial blood pressure under sodium pentobarbital anesthesia was 133 mm Hg and 146 mm Hg, for the test and the control dogs, respectively. On gross examination, all the kidneys appeared normal, and the renal architecture showed no histologic abnormality.

Comment

The data of the study are listed in *Table 2*. It was not possible to distinguish the kidneys of the test dogs from those of the control dogs, on the basis of the functional studies, except for traces of albumin in the urine of two of the test dogs, one of which had a positive urine culture. There was no evidence that an anatomic venous obstruction was present as the cause for proteinuria from those two particular kidneys. Urinary protein excretion was detected both in control and in test animals (*Table 3*) at approximately three months after renal reimplantation. This proteinuria could be attributed to reversible renal damage resulting from a period of ischemia which occurred during reimplantation of the kidneys, as this was observed in both groups of dogs and averaged 1.75 g per liter for the control dogs, and 1.74 g per liter for the test dogs. The exogenous creatinine clearance in all dogs tested was compatible with the expected clearance of one normal kidney. The blood pressures of both groups of dogs were normal as measured under pentobarbital anesthesia.

All kidneys appeared normal on histologic examination and there was no

Table 2.—Summary of results of study of 6 treated autograft canine kidneys and 2 control autograft canine kidneys

Dog no.	Blood urea nitrogen, mg/100 ml	Serum creatinine, mg/100 ml	Creatinine clearance, ml/kg of body weight/min	Mean arterial blood pressure, mm Hg	Urine	
					Culture	Protein content
Test dogs						
224 K	21	0.9	—	150	Positive	Trace
247 K	14	—	—	130	Negative	Negative
288 K	14	1.4	3.5	135	Negative	Trace
298 K	14	1.3	3.7	91	Negative	Negative
301 K	14	1.0	—	133	Negative	Negative
306 K	19	1.4	2.4	119	Negative	Negative
Control dogs						
157 K	13	1.3	3.36	138	Negative	Negative
163 K	19	—	—	155	Negative	Negative

Table 3.—Urine protein content at three months' postreimplantation of autograft kidney

Dog no.	Urinalysis
	Protein, g/liter
Test dogs	
224 K	1.66
247 K	2.80
288 K	4.64
298 K	0.60
301 K	0.25
306 K	0.48
Control dogs	
157 K	1.50
163 K	2.00

evidence of microinfarcts that might have been caused by emboli of lipid aggregates during the perfusion, as has been described by Belzer and associates.[5] The special processing of the plasma is believed to be the important factor in preventing this cause of renal damage.

Summary and conclusion

Six dogs each with a solitary autograft kidney, which had been preserved for 24 hours extracorporeally by hypothermic pulsatile perfusion of cryoprecipitated homologous plasma, were observed for a period of approximately one year after autotransplantation and immediate contralateral nephrectomy.

After this prolonged period of time the functional characteristics and histologic appearance of the preserved kidneys were essentially the same as those of the kidneys from the two control animals. These studies indicate that canine kidneys can be successfully stored extracorporeally for a 24-hour period by this method of preservation, with subsequent long-term autograft survival and sustained normal renal function.

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