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IMPROVED PORCINE RENAL PRESERVATION WITH A SIMPLE EXTRACELLULAR SOLUTION—PBS140

COMPARISON WITH HYPEROSMOLAR CITRATE AND UNIVERSITY OF WISCONSIN SOLUTION

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In this prospective randomized trial a porcine model of renal autotransplantation was used to compare quality of preservation, as reflected by detailed analysis of posttransplant renal function, following 24-hr cold storage in phosphate-buffered sucrose (PBS140), hyperosmolar citrate (HOC), and University of Wisconsin (UW) preservation solutions. There were 6 deaths with primary nonfunction: 3 of 5 HOC, 2 of 5 UW, but only 1 of 5 PBS140. Analysis of the whole group and separate analysis of the survivors demonstrated significantly better renal function following preservation with PBS140 when compared with both HOC and UW, with a lower peak serum creatinine ($P=0.02$) and improved loop of Henle function ($P=0.02$). The animals in the PBS140 group also demonstrated a more rapid return to normal creatinine, higher GFR, improved tubular function, and higher effective renal plasma flow, with figures approaching statistical significance ($P=0.06-0.07$). The proposal of UW as a universal storage medium prompted this study, and its results suggest the need for a clinical comparison of renal preservation using UW and PBS140 in a prospective randomized trial.

The University of Wisconsin Solution (UW) has been promoted as a universal storage medium, suitable for the prolonged preservation of all intraabdominal organs. Although the results of liver and pancreas preservation have shown improvement and extended storage times (1-4), the published data on renal preservation remain less impressive (5).

In this prospective randomized study we have used a porcine autograft model of renal transplantation following 24-hr cold-storage preservation to compare the post-transplant function

of kidneys preserved in UW with kidneys preserved in hyperosmolar citrate solution (HOC) and in phosphate-buffered sucrose (PBS140).^{*} A 14-day follow-up period with detailed assessment of renal function was used to assess the severity of storage injury that occurred in each part of the nephron.

MATERIALS AND METHODS

Preservation solutions. Standard 1-L bags of UW (Belzer UW-CSS made by NPBI, The Netherlands) were kindly provided by DuPont Pharmaceuticals, U. K. HOC (Travenol Kidney Perfusion Solution) was purchased from Travenol Laboratories Ltd., England, also in 1-L bags. PBS140 was manufactured by our own hospital pharmacy as previously described (6) and supplied in 1-L bottles. Table 1 illustrates the major components of these solutions.

Experimental methods. Three groups of five pigs were studied. Female nonpregnant Landrace-Large White hybrids between 4 and 8 weeks of age and weighing 20-45 kg were used. The animals were kept in clean pens (2×3-m) and were allowed to become accustomed to their surroundings for at least 7 days before any operative procedures were carried out. They were fed ad libitum with supergrade pig pellets and drinking water.

Study design: The pigs were randomized into three groups, according to the preservation solution used: (1) UW group (n=5); (2) HOC group (n=5); (3) PBS140 group (n=5).

Anesthesia and intraoperative care: General anesthesia was induced with halothane 2.0-5.0% and oxygen 8 L/min via a mask, with atropine 0.6 mg intramuscularly, followed by diazepam 10-20 mg intravenously prior to intubation and ventilation. Anesthesia was maintained with halothane 0.5-1.0%, oxygen 2-4 L/min, nitrous oxide 2-4 L/min, fentanyl 0.5-1.0 mg i.v., ketamine 10 mg/kg/hr i.v., and thiopentone in small doses if necessary. The animals underwent left nephrectomy on day -1, and right nephrectomy followed by left renal autotransplan-

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^{*} Abbreviation: PBS140, phosphate-buffered sucrose solution.

tation on day 0 (postoperative days were numbered 1–14). Intraoperative monitoring was carried out by means of an electrocardiogram, and an arterial blood pressure line inserted into the superficial femoral artery. Mannitol 0.5 g/kg and heparin 5000 units i.v. were administered during the 15 min immediately prior to clamping of the left renal vessels and again in the 15 min immediately prior to reperfusion. During the operative procedures each animal received 1.0 L 4% dextrose, 0.18% saline, and 0.5–1.0 L 10% dextrose i.v. (according to weight). Postoperatively 1.0–2.0 L 4% dextrose and 0.18% saline were given i.v. on days 1 and 2. Buprenorphine (Temgesic) was administered i.v. and i.m. for analgesia as required during the postoperative period. Antibiotic prophylaxis was given as follows: gentamicin 40 mg and ampicillin 1 g i.v. on the operative days followed by gentamicin 40 mg 12-hourly on day 1, with ampicillin 1 g 12-hourly on days 1 and 2.

Operative procedures. Day –1: A central venous line was placed into the external jugular vein and tunneled posteriorly, and a sampling line attached. A left rectus muscle-splitting incision was made, and an extraperitoneal dissection of the kidney carried out. After mannitol and heparin administration, the renal vessels were clamped and divided, the kidney weight was recorded, and the renal artery was cannulated for flush cooling—using 800 ml UW, PBS140, or HOC, at approximately 4°C, infused from a height of 100 cm. During cooling the core temperature of the kidney was monitored using a fine thermistor probe inserted into the upper pole to a depth of 0.5–1.0 cm. The kidney was reweighed and then stored for 24 hr (in double plastic bags on ice) in a further 200 ml of the flush solution. The abdomen was closed after ligation of the renal vessels.

Day 0: Through a right rectus-splitting incision and using an extraperitoneal approach, the right kidney was removed. The left kidney was weighed once more on removal from the storage medium; and, following administration of heparin, anastomosis of the renal vein was end-to-side to the external iliac vein and the renal artery end-to-side to the common iliac artery. On completion of the venous anastomosis mannitol was given as described above.

Following reperfusion, renal blood flow was monitored every 5 min for 1 hr using an electromagnetic flow meter (Statham Blood Flowmeter SP2204, Gould Inc., Medical Products Division, Oxnard, CA). A small incision in the peritoneum allowed delivery of the bladder for catheterization and ureteroneocystostomy prior to abdominal closure.

Postoperative monitoring. Renal function was assessed by daily serum and urine urea, creatinine, and electrolyte measurements. Three times weekly a detailed analysis of renal function was carried out to examine the function of the individual parts of the nephron as follows: (1) glomerular filtration rate—inulin clearance (7); (2) tubular function—sodium and lithium reabsorptions (8); (3) loop of Henle function—urine and serum osmolalities to calculate the concentrating

ability as free water clearance (9); (4) effective renal plasma flow—paraaminohippurate (PAH) clearance (10).

Surviving animals were sacrificed after 14 days.

Statistical analysis of results. The results were analyzed by a one-way analysis of variance. Where the data did not readily lend themselves to a parametric analysis, a chi-squared approximation to the distribution of the Kruskal-Wallis test statistic was computed using a nonparametric one-way analysis procedure. For nonparametric analyses, the analysis was repeated using a Mann-Whitney *U* test on each of the individual comparisons. A *P* value of less than 0.05 was taken to be significant.

The parameters analyzed were:

- (1) For all animals: maximum value and time to maximum value (T_{max}) for serum urea and creatinine.
- (2) For surviving animals: time to return to normal (T_{crit}) and area under curve (AUC) for serum urea and creatinine (calculated by square approximation). The critical values (T_{crit}) were defined as the time to urea <10 mmol/L and creatinine <200 μ mol/L.
- (3) Maximum and first and second week results for the detailed renal function tests.

RESULTS

Pretransplant data. Table 2 summarizes the pretransplant data. All groups were similar in terms of pig and kidney weight, total ischemic time, and anastomosis time. Figure 1 illustrates the core temperatures of the kidneys during cooling.

Change in kidney weight during flush and storage The kidneys in the UW group lost a significant amount of weight during flush prior to storage (Fig. 2), whereas the kidneys in the other two groups gained weight. Following 24-hr storage the percentage weight loss was similar in all three groups of kidneys.

Renal blood flow. The mean renal blood flow during the first hour following reperfusion was above 1 ml/min/g in the PBS140 group throughout the first 60 min posttransplant and below 1 ml/min/g in the UW group (Fig. 3), but the differences were not significant.

Deaths in renal failure. Several animals died with primary nonfunction in the absence of technical problems: 1 in the PBS140 group, 3 in the HOC group, and 2 in the UW group. The subsequent results therefore largely relate to the survivors—only 2 animals in the HOC group and 3 in the UW group, but 4 in the PBS140 group.

Glomerular filtration. Figure 4 illustrates the daily serum creatinine results for the three groups of pigs. Further data are shown in Table 3. The maximum value and T_{max} for serum creatinine in the PBS140 group was significantly lower than animals in both the HOC group and the UW group (maximum: $P=0.02$, T_{max} : $P=0.02$). Although the other comparisons for serum creatinine and for serum urea did not achieve statistical significance, the trend in the results was consistent, with the PBS140 group providing the smallest maximum value ($P=0.06$) and T_{max} for serum urea, and the smallest AUC for both creatinine and urea.

TABLE 1. Preservation solutions

	HOC	PBS140	UW
Impermeants	Mannitol	Sucrose ^a	Raffinose Lactobionate
Colloid	—	—	Pentafraction
Buffers	Citrate	Phosphate ^b	Phosphate
Na ⁺ (mmol/L)	80	120	30
K ⁺ (mmol/L)	80	—	80
Metabolic additives	Magnesium Sulfate	—	Magnesium Sulfate Adenosine Allopurinol Glutathione
Osmolarity (mOsm/L)	400	310	320
pH	7.0	7.2	7.4

^a Sucrose 140 mmol/L.
^b Phosphate 60 mmol/L.

TABLE 2. Pretransplant data

	HOC	PBS140	UW
Pig weight (kg)	30.8±2.5	37.7±1.8	30.5±2.3
Kidney weight (g)	125.7±17.2	142.2±9.0	133.5±9.7
Ischemic time (hr)	22.7±1.1	22.7±1.1	24.9±0.8
Anastomosis time (min)	30.8±1.2	30.2±2.4	31.2±1.2

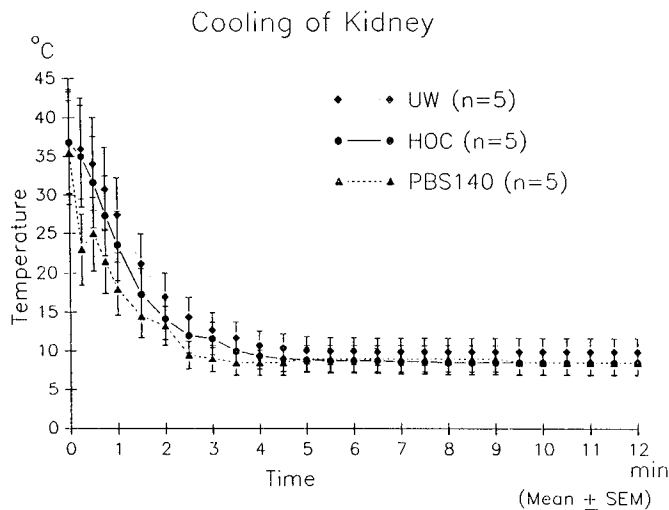


FIGURE 1. The core temperature of the kidneys during cooling by the flush with HOC, PBS140, or UW previously stored at 4°C.

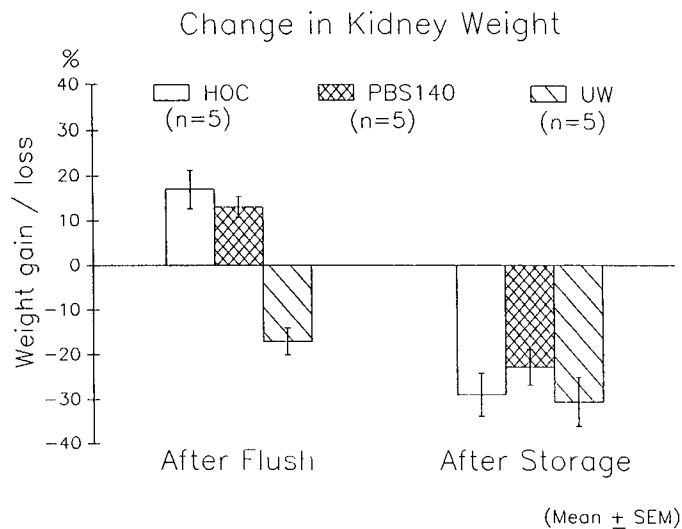


FIGURE 2. The weight gain and loss following the flush with HOC, PBS140, or UW; and following 24-hr cold storage in the preservation solutions. Weight change expressed as percentage gain or loss compared with preflush wet weight of the kidneys.

Glomerular filtration rate was measured three times weekly and compared for all surviving animals at the end of the first and second weeks (Table 3). Although the animals in the UW group achieved the highest GFR by the end of the first week posttransplant (not significant: $P=0.61$), there was no further improvement, and by the end of the second week the animals in the PBS140 group had achieved the highest values for GFR, approaching statistical significance ($P=0.06$). The apparent early achievement of an adequate GFR in the UW group was not reflected in the serum urea and creatinine results, and analysis of the early function of the UW animals indicated that renal function had not recovered until the end of the first posttransplant week (GFR: day 1 = 1.2 ± 0.8 ml/min; day 3 = 3.2 ± 2.1 ml/min), whereas the PBS140 group showed good evidence of recovery from day 1 (GFR: day 1 = 27.9 ± 2.6 ml/min [$P=0.04$]; day 3 = 23.1 ± 6.2 ml/min [not significant]).

Tubular function. The recovery of tubular function was more rapid in the PBS140 group, as indicated by the return of

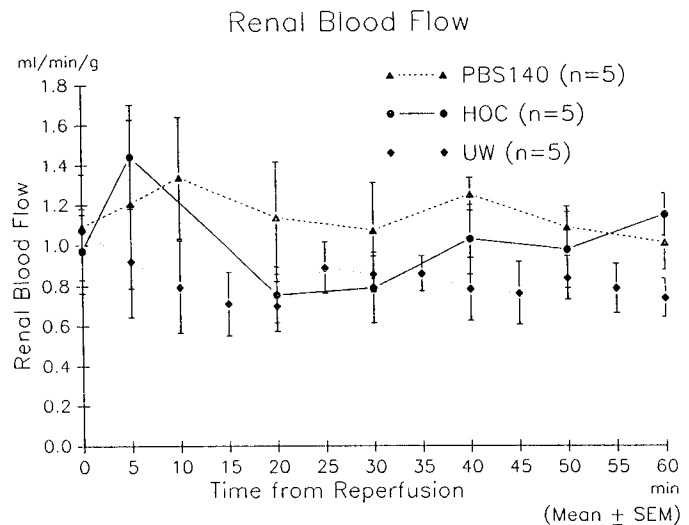


FIGURE 3. The renal blood flow during the first 60 min following reperfusion of the kidneys with blood, (expressed as milliliters per minute per gram of renal tissue) calculated from the preflush wet weight of the kidneys at nephrectomy.

fractional sodium and lithium reabsorptions to within normal limits by the end of the first week posttransplant (Table 3). Although the results did not achieve statistical significance, the values for lithium reabsorption were highest in this group at the end of the first and second weeks.

Loop of Henle function. The ability to concentrate urine (as indicated by negative free water clearance) in the PBS140 group was increasingly more powerful throughout the follow-up period when compared with the UW group, which had no concentrating ability at all at the end of the first posttransplant week (Table 3). The maximum loop of Henle function achieved in the PBS140 group was significantly greater than both the HOC group and the UW group ($P=0.02$) and this remained significantly greater at the end of the two-week follow-up period ($P=0.03$).

Effective renal plasma flow. There was a more rapid return to normal ERPF (PAH clearance) in the PBS140 group, and there was continued improvement in this group such that by the end of the second week posttransplant the difference in ERPF was approaching statistical significance ($P=0.07$) when compared with the animals in the HOC and UW groups (Table 3).

DISCUSSION

Experimental findings in renal preservation are based upon the assumption that the result obtained in one mammalian species may be generalized to others, including man. However, there may be significant interspecies differences in the efficacy of preservation solutions, as has been suggested by studies of warm ischemia (11). The use of the pig as an experimental model for renal preservation research has considerable merit, since porcine kidneys are more like those of man than those of the classically chosen dog in terms of development, anatomy, and physiology (12, 13). One further advantage of the pig is that it provides a more sensitive model for preservation experimentation: successful preservation of porcine kidneys by cold storage for more than 24 hr has rarely been reported (14) (compared with the regular achievement of 72-hr preservation

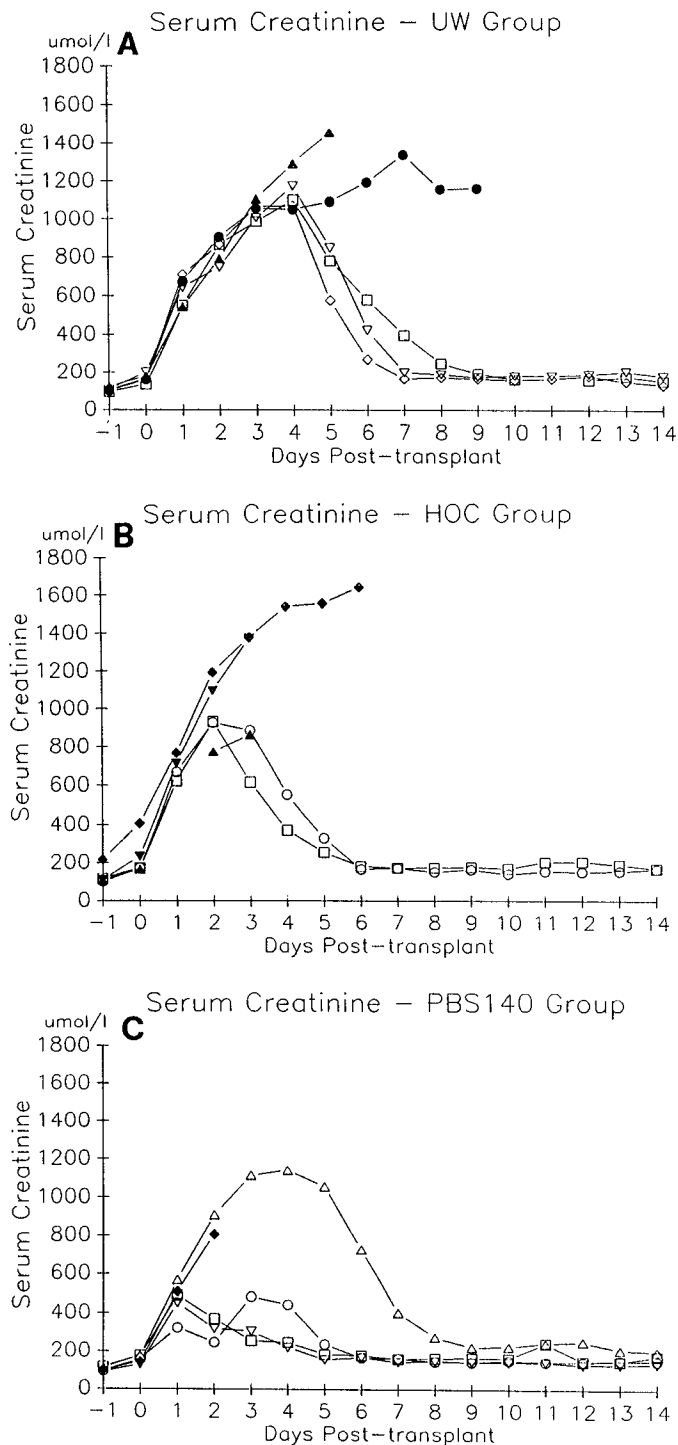


FIGURE 4. The serum creatinine of the animals during the 14-day follow up period following the autotransplant, (expressed as $\mu\text{mol/L}$). Survivors are represented by open symbols and nonsurvivors by solid symbols; (a) UW group; (b) HOC group; (c) PBS140 group.

in the dog [15, 16]), and the success rates of porcine 24-hr cold storage in our own department have been low. For this work, therefore we limited the storage time to 24 hr. We must conclude, however, that it is not known which animal model best reflects the clinical situation: the porcine model may be too sensitive and the canine model not sensitive enough.

As in any large animal study, the number of animals entered

into this trial was small, but the groups were well standardized in terms of pig and kidney weight, total ischemic times, cooling prior to preservation, and anastomosis times. The kidneys in the UW group cooled slightly more slowly than the other two groups because of the high viscosity of UW; and although UW is isoosmolar the kidneys in this group lost a significant amount of weight within the flush period (15–20 min) prior to storage. This is probably an effect of the colloid component (hydroxyethyl starch pentafraction), added to prevent cellular swelling; the lactobionate and raffinose may also be contributory (17). The weight loss was more gradual in the kidneys preserved in PBS140 and HOC: following the 24-hour preservation period all three groups of kidneys had lost a similar amount of weight.

The renal blood flow during the first hour posttransplant has been documented in dogs, with a figure of less than 1 ml/min/g at 1 hr correlating with a poor chance of immediate graft function (18). The data published for pigs are scant, but it is interesting that the mean flows in the UW animals were consistently below this figure and the PBS140 group consistently had mean flows above this figure.

The posttransplant renal failure rates and functional data from this study demonstrate that the 24-hr preservation of porcine kidneys with PBS140 was better than that achieved with either HOC or UW. Only 2 of the 5 animals in the HOC group survived, and only 3 of the 5 UW group, compared with 4 of the 5 in the PBS140 group. Furthermore, examining the data from the animals that survived, the overall renal function in the PBS140 group was improved; and despite the small numbers of survivors in the HOC and UW groups the comparisons revealed significantly worse results when compared with the PBS140 group, confirmed by a strict statistical analysis. The animals in the PBS140 group achieved a higher GFR (with a significantly lower peak plasma creatinine and T_{max}), a rapid return to normal tubular function, and improved loop of Henle function as well as a higher effective renal plasma flow.

The simplicity of PBS140 compared with the complicated (and therefore expensive) composition of UW makes PBS140 an attractive alternative for renal preservation, although its value has yet to be proved in the storage of other organs. This simplicity, however, makes it difficult to explain the advantages conferred over UW, at least in the pig. The major difference, which may be effective in this regard, is that PBS140 has a high sodium content and no potassium, and it is therefore classed as an extracellular preservation solution. It is interesting that recent work using UW with a reversed sodium-potassium ratio for canine liver, kidney, and pancreas preservation has yielded results at least as good as the classic high-potassium UW (19), and the authors commented that the use of such an extracellular solution for human liver preservation would obviate the on-table flush currently needed prior to reperfusion. The concentration of the components of PBS140 appears to be important, as previous work from our laboratory has demonstrated significantly improved renal preservation of 72-hr cold-stored canine kidneys compared with HOC (16), but Belzer's results with 48-hr preservation of canine kidneys were poor (15) using phosphate-buffered sucrose with a lower sodium and sucrose content (20).

The recent Euro-Transplant multicenter trial of renal preservation using UW versus Euro-Collins solution (21) has the failing that EC is no longer a good "gold standard" for comparison, and it is not surprising that the kidneys preserved in UW have achieved a lower rate of requirement for postoperative

TABLE 3. Summary of posttransplant results

	HOC	PBS140	UW
Number in group	5	5	5
Survivors	2	4	3
Peak creatinine ($\mu\text{mol/L}$)	1139 \pm 156	671 \pm 128 ^a	1225 \pm 74
AUC creatinine (μmol)	4792 \pm 172	4234 \pm 1103	6394 \pm 197
T _{max} creatinine (days)	3.4 \pm 0.7	2.0 \pm 0.4*	5.0 \pm 1.0
T _{crit} creatinine (days)	6.0 \pm 0.0	7.5 \pm 2.2	9.0 \pm 1.5
Peak urea (mmol/L)	56.7 \pm 12.8	32.6 \pm 9.1***	75.8 \pm 14.8
AUC urea (mmol)	206.4 \pm 6.5	202.0 \pm 59.0	338.2 \pm 14.7
T _{max} urea (days)	3.4 \pm 0.7	2.8 \pm 0.7	5.0 \pm 1.0
T _{crit} urea (days)	6.0 \pm 0.0	6.5 \pm 1.3	9.3 \pm 0.9
Week 1:			
GFR (ml/min)	15.4 \pm 5.0	41.0 \pm 18.2	64.1 \pm 44.9
Sodium reabsorption (%)	99.2 \pm 0.3	98.8 \pm 1.0	74.4 \pm 24.8
Lithium reabsorption (%)	76.5 \pm 0.0	84.4 \pm 4.7	56.0 \pm 29.2
Free water clearance (ml/min)	-0.37 \pm 0.76	-0.33 \pm 0.38	+0.06 \pm 0.26
ERPF (ml/min)	353 \pm 110	557 \pm 129	135 \pm 51
Week 2:			
GFR (ml/min)	44.0 \pm 1.6	106.4 \pm 22.4***	50.9 \pm 5.3
Sodium reabsorption (%)	99.6 \pm 0.2	99.8 \pm 0.1	99.8 \pm 0.1
Lithium reabsorption (%)	81.6 \pm 3.4	85.1 \pm 1.1	74.6 \pm 7.6
Free water clearance (ml/min)	-0.24 \pm 0.10	-1.55 \pm 0.26**	-0.25 \pm 0.15
ERPF (ml/min)	317 \pm 129	880 \pm 133	134 \pm 38
Maximum:			
GFR (ml/min)	89.1 \pm 6.9	106.4 \pm 22.4	86.5 \pm 34.5
Sodium reabsorption (%)	99.9 \pm 0.1	99.9 \pm 0.0	99.9 \pm 1.0
Lithium reabsorption (%)	82.8 \pm 4.6	90.6 \pm 2.7	97.0 \pm 2.3
Free water clearance (ml/min)	-0.85 \pm 0.29	-1.55 \pm 0.26*	-0.28 \pm 0.13
ERPF (ml/min)	466 \pm 148	1299 \pm 388	310 \pm 120

^a P values: * 0.02; ** 0.03; *** 0.06.

dialysis. Retrospective analysis of human kidneys transplanted at our own hospital has demonstrated HOC to give a significantly higher rate of primary renal function than EC (22), and preliminary studies using PBS140 in a prospective randomized trial at our unit have shown the rate of delayed primary function to be reduced even further when compared with HOC (23). The proposal of UW as a universal storage medium prompted this study, and its results suggest the need for a clinical comparison of renal preservation using UW and PBS140 in a prospective randomized trial.

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SIGNIFICANCE OF BIOCHEMICAL MARKERS IN EARLY DETECTION OF CANINE LUNG ALLOGRAFT REJECTION¹

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To evaluate the significance of bronchoalveolar lavage fluid, levels of tumor necrosis factor- α (TNF), gamma-interferon, interleukin 2, and soluble IL-2 receptor in early detection of canine lung allograft rejection, bronchoalveolar lavages were performed serially in mongrel dogs before and after single lung transplantation. The dogs were divided into three groups. Group 1 (control group) consisted of one in which neither donor nor recipient dogs were treated with cyclosporine. In group 2 (CsA-pretreated group) only donors were treated with CsA orally at a single dose of 20 mg/kg/day for 3 days prior to single lung transplantation. In group 3 only recipients were treated with CsA orally at a single dose of 20 mg/kg/day for a short period of 9 days after single-lung transplantation. Marked elevation was found of TNF, IFN- γ , IL-2, and IL-2R in BALF obtained from the grafted lungs in group 1 and group 2 dogs. The levels of these markers were significantly higher than those obtained from the normal, native lungs ($P < 0.05$). Two of three recipients in group 2 had pneumonia in the native lungs on day 10 after single-lung transplantation. All markers except IFN- γ in BALF obtained from the infected native lungs were also increased, but the titers were less than those obtained from the grafted lungs at the same time. There were significantly higher levels of TNF, IL-2, and IL-2R present in the BALF of grafted lungs of dogs in group 1 than group 2 ($P < 0.05$). In group 3, BALF levels of these markers from the grafted lungs were not significantly different from those of the normal and native lungs during the period of CsA treatment after single-lung transplantation. On various days after discontinuation of CsA treatment, BALF levels of all markers began to rise. Abnormal levels of BALF markers obtained from the grafted lungs heralded the appearance of abnormalities detected by chest x-ray films.

Our study suggests that serially measuring BALF levels of TNF, IFN- γ , IL-2, and IL-2R may serve as a useful means in monitoring the immunologic status of canine lung allografts and in the early detection of lung allograft rejection. The role of BALF IFN- γ in distinguishing lung allograft rejection from pulmonary infection needs further studies.

Up to 1980, the outcome of lung transplants had been very poor. The clinical outlook of single lung transplantation has changed quite substantially during the past years, particularly due to the efforts of J.D. Cooper and the Toronto Lung Transplant Group. There, effective and almost uniformly successful single-lung transplantation has been achieved (1). However, there remains a further obstacle, that of diagnosing accurately episodes of rejection and of distinguishing such events from pulmonary opportunistic infections (2). Open-lung biopsy was advocated as the "gold standard" for the diagnosis of lung allograft rejection clinically and experimentally (3, 4). In recent years, several studies have indicated that transbronchial biopsy is of certain value in the diagnosis of lung allograft rejection (5-11).

Immunologic monitoring that provides relevant and early information with respect to ongoing allograft rejection would certainly facilitate clinical decisions. Studies with monoclonal antibodies to cell-surface antigens have shown that T cells and mononuclear phagocytes predominate in the rejecting graft (12, 13). Evaluation of cytokines seems to represent the most promising candidate for successful monitoring because they are released from immune T cells and macrophages, almost immediately after antigen recognition, and they are known to play a crucial role in amplification of alloreactivity.

Among the first events of T cell activation are the synthesis and surface expression of a receptor for IL-2. IL-2R is released into the supernatant by activated T cells during in vitro stimulation with mitogens or antigens (14, 15). IL-2 is released from the activated T cells and required for the initiation and maintenance of both T and B cell responses. TNF is released

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