INVESTIGATIVE NUCLEAR MEDICINE

Scintigraphic Evaluation of the Viability of Cold-Preserved Kidneys Before Transplantation

D. Anaise, K. Sato, H. Atkins, Z. Oster, H. Asari, W. Waltzer, W. Pollack, R. Bachvaroff, and F. Rapaport

State University of New York at Stony Brook, New York

Renal microcirculation studies utilizing microspheres can provide a highly reliable index of the viability of preserved kidneys. We describe a noninvasive method that permits subsequent transplantation of each tested kidney. Fresh canine kidneys were removed and perfused with either Collins' solution or Collins' solution with 5 mg/l of trifluoperazine (TFP), and preserved at 4° to 6°C in their respective perfusates for 5, 24, 48, or 72 hr. At the end of each period, the preserved kidney was perfused with 50,000 Tc-99m-labeled microspheres. Uniform cortical activity occurred in all kidneys preserved for 5 hr, with moderate decreases in cortical activity at 24 hr. Kidneys perfused with Collins' solution showed progressively decreasing cortical flow with sparse cortical activity after 72 hr. In kidneys perfused with Collins' solution containing TFP, however, cortical flow remained relatively well preserved at 48 and 72 hr. Direct correlation between viability of the cortical microcirculation and the capacity of the corresponding kidney to sustain life upon retransplantation into the original host was observed.

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One of the major problems in kidney preservation has been the lack of availability of in vitro tests of viability capable of providing a reliable index of posttransplant function. Such techniques would be of significant value in permitting the surgeon to select and use only those kidneys that will maintain adequate function after transplantation. They could also increase utilization of procured kidneys considered nonviable by currently existing criteria. A number of such tests have been described, including: (a) analysis of hemodynamic behavior of the preserved kidney; (b) accumulation of intracellular enzymes in the perfusates; (c) glomerular and tubular function; and (d) microscopic changes, etc. None of these tests, however, can provide an accurate index of posttransplantational function (*1*).

The ease with which hemodynamic measurements can be made in the cold-perfused kidney has led to widespread use of this test for the assessment of kidney viability. Several studies have shown good correlation between changes in perfusate flow, increased vascular resistance, and subsequent transplant function (*2,3*). It is generally agreed, however, that such criteria are not fully reliable (*4,5*)—particularly because determinations of the total renal blood flow do not take into account the complexities of the renal microcirculation, with particular regard to its inherent autoregulation and to the possible intrarenal redistribution or shunting of blood flow from cortex to hilum as a consequence of ischemic injury.

In further studies of this problem, Miller et al. (*6*) approached the question of redistribution of blood flow in preserved kidneys by a microsphere distribution technique. They showed that kidneys exposed to 30 min of warm ischemia exhibited poor blood flow in the outer renal cortex, with increased blood flow in the juxtamedullary cortex. Such kidneys also failed to function upon retransplantation.

For reprints contact: David Anaise, MD, Transplantation Service, Dept. of Surgery, Health Sciences Ctr., State Univ. of New York, Stoney Brook, NY 11794.

TABLE 1. CORTEX ACTIVITY RATIOS IN CANINE KIDNEYS PERFUSED WITH COLLINS' AND COLLINS' PLUS TRIFLUOPERAZINE SOLUTIONS AT VARYING DURATIONS OF PRESERVATION

Hours of preservation	Perfusate	No. of animals	Perfusion flow cc/min	Cortex activity ratio %	p value*
5	Collins	5	12.0 ± 1.0	52.2 ± 6.4	NS
5	C + TFP†	5	19.0 ± 5.0	51.0 ± 4.1	
24	Collins	6	17.5 ± 1.8	46.0 ± 8.9	NS
24	C + TFP	5	12.6 ± 5.5	48.0 ± 6.4	
48	Collins	6	15.7 ± 3.5	38.0 ± 13.6	<0.05
48	C + TFP	5	15.6 ± 3.6	48.2 ± 4.3	
72	Collins	7	9.1 ± 4.9	24.6 ± 12.7	<0.02
72	C + TFP	7	14.1 ± 4.8	45.5 ± 5.6	

* Data analyzed statistically by Student's t-test.
† Collins plus tripfluoperazine.

Recent technical advances have produced microspheres of predetermined small diameters, and these are particularly useful in further studies of the microcirculation of the kidney (*7–10*). These methods have, however, precluded transplantation of the kidney upon completion of the study, because they require injection of the radiolabeled microspheres into the kidney, followed by sectioning of the kidney into thin slices for measurement of the radioactivity of each sample in a gamma counter or by autoradiography (*11*). The present study describes the utilization of biodegradable radiolabeled microspheres for noninvasive assessment of viability of a cold-stored kidney without interference with the subsequent transplantability of the total organ. The method involves the scintigraphic mapping and quantification of such labeled microspheres after injection, thus eliminating the need for kidney sections. The results indicate that this technique provides a reliable index of organ viability before transplantation. The data also highlight the potential value of a calmodulin inhibitor, trifluoperazine (TFP), in increasing the resistance of the canine kidney to the effects of cold preservation (*12*).

MATERIALS AND METHODS

Twenty-four adult male and female mongrel dogs weighing 15–25 kg were used. Each animal was explored under general anesthesia, and both kidneys were removed by atraumatic sharp dissection. The left kidney was flushed immediately with cold Collins' solution (*13*) (Group A), and the right kidney was flushed with Collins' solution to which 5 mg/l of trifluoperazine (TFP) (*14*) had been added (Group B). Perfusion was performed by gravity, with a pressure head of 100 cm, using a tapered catheter placed in the renal artery. The perfused kidneys were stored under sterile conditions at 4°–6°C for 5, 24, 48, or 72 hr. The kidneys in each group were imaged scintigraphically through a 3-mm pinhole collimator under sterile precautions (Fig. 1a). Each test was performed at the time of completion of the required period of cold preservation (5, 24, 48, or 72 hr) (Table 1 indicates the number of kidneys studied at each time interval).

For the purposes of this study, 50,000 biodegradable albumin microspheres measuring 20 μ in diameter, labeled with 500 mCi of Tc-99m pertechnetate, were suspended in 100 cc of Collins' solution. The suspension was agitated by a sonic device, and was perfused through the renal artery of each kidney from a height of 100 cm, the flow rate being recorded. Imaging was performed with a gamma camera using a 3-mm pinhole with a 20% symmetrical window at 140 keV. Data in a 64 × 64 matrix format were recorded on the magnetic disk of a minicomputer with the kidney placed in the center of the field of view at 6–8 cm from the crystal face. Two hundred thousand counts were obtained over a period of 1–4 min. No significant falloff in counts was detected at the periphery of the kidneys. After data acquisition, the

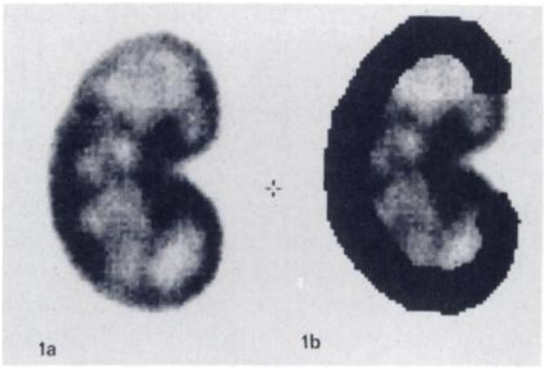

FIG. 1. Canine kidney flushed with Collins' solution, injected with 50,000 Tc-labeled microspheres and scintigraphed under sterile conditions. Note cortical acitvity (1a). Activity of microspheres trapped in cortex is measured by placing an irregular region of interest over renal cortex. Cortical activity is expressed as fraction of total activity of kidney (cortex acitvity ratio, CAR) (1b).

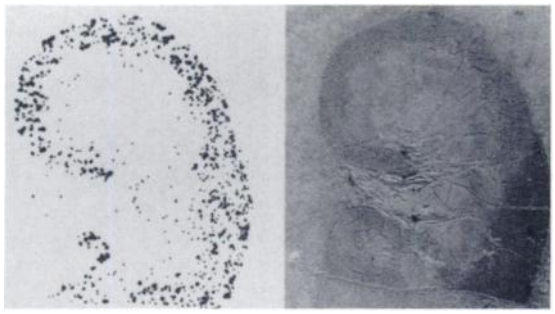

FIG. 2. Autoradiogram and anatomical slice of kidney shown in Fig. 1. Note trapping of microspheres in renal cortex.

FIG. 3. Collins' solution preservation for 48 hr. Note further decrease in cortical activity and some further increase in hilar activity.

kidney image was displayed on the computer screen and regions of interest (ROI) were placed over the whole kidney and over the cortex. Total kidney activity and kidney cortical activity were determined (Fig. 1), and the cortical activity ratio (CAR) was calculated as follows: CAR = [100x] cortical activity/total kidney activity. Distribution of the microspheres was studied further by autoradiography (Fig. 2) and gamma counting of each kidney section, and the results were compared with the distribution of microspheres obtained by scintigram. These studies showed that the microspheres are trapped in the cortex and outer medulla. Serial anatomical studies were done on sagittal cuts of these kidneys; this revealed that the width of this anatomical region comprises 25% of the width of the whole kidney (Fig. 2). This fact was found to be particularly useful in plotting the region of interest. While in the majority of the cases the selection of the cortex can be done visually and can be enhanced by increasing the lower threshold, the limiting of the ROI to 25% of the width of the whole kidney (Fig. 2) enhanced the reliability and accuracy of this selection (Fig. 1b). Finally, all cases were analyzed independently by three of the authors (H.A., Z.O., and D.A.) to reduce bias and increase the reproducibility of this technique.

Further studies were conducted to ascertain the sensitivity and safety of scintigraphy in predicting posttransplant viability. For this purpose, seven adult female mongrel dogs were explored under general anesthesia, and the left kidney was removed and preserved in the cold under sterile conditions for 72 hr. The kidneys were then perfused with microspheres and were imaged as in the previous experiment. Each kidney was then transplanted into the groin of the original host. Contralateral nephrectomy was performed 3 wk later, and kidney function was assessed for the following 90 days. Analysis of the regional distribution of microspheres was performed by one of us (H.A.), without any knowledge of the posttransplant function of each of these kidneys. A correlation was then made between the cortical activity ratios and the actual ability of each kidney tested to sustain life after transplantation.

RESULTS

Twenty-four kidneys perfused with Collins' solution were preserved at 4° to 6°C for 5, 24, 48, or 72 hr, respectively. As shown in Fig. 1a, kidneys preserved for 5 hr showed a uniform distribution of microspheres throughout the cortex, with a mean CAR of 52.2%. Slight decreases in cortical activity, and a rise in radioactivity in the hilum, occurred in organs preserved for 24 hr. Kidneys preserved for 48 hr showed only patchy uptake of microspheres in the cortex, with a mean CAR of 38% (Fig. 3).

At 72 hr, cortical uptake was poor (mean CAR = 24.6%), with trapping of most injected microspheres distributed throughout the kidney (Fig. 4A).

The regional distribution of microspheres was essentially similar in kidneys preserved in Collins' solution, with or without TFP, after 5 and 24 hr, however, of cold preservation. Significant differences occurred when Collins'-TFP solution was used to perfuse and preserve kidneys for 48 and 72 hr. As shown in Figs. 4B and 5, there was a relatively uniform distribution of microspheres (mean CAR values = 48.2% at 48 hr, and 45.5% at 72 hr) when TFP was added to the flush solution, in

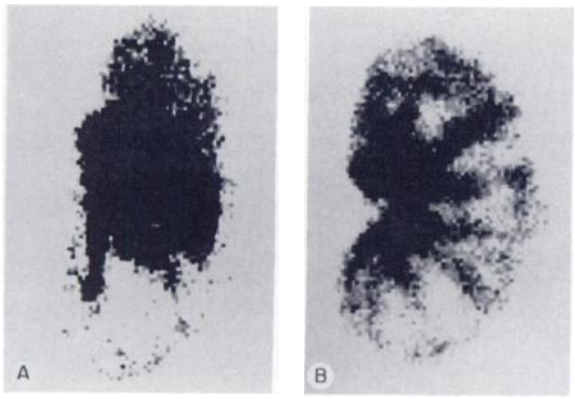

FIG. 4. Kidneys flushed with Collins' solution (A) or Collins' plus trifluoperazine (B) and preserved for 72 hr at 4°C. Note preservation of cortical flow in TFP-treated group.

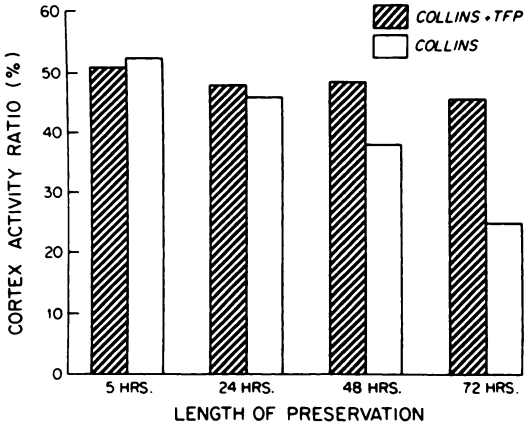

FIG. 5. Preservation of renocortical blood flow with and without TFP (cf. Table 1). Activity of microspheres in cortex is compared with total activity and expressed as mean cortex activity ratio (CAR). Preservation times of 48 and especially 72 hr produced marked decreases in cortical activity in kidneys preserved with Collins' solution alone. Cortical activity persisted, however, in kidneys preserved for same period with Collins' solution and TFP.

contrast with the CAR values of 38% at 48 hr and 24.6% at 72 hr observed when Collins' solution was used alone.

Contrasting with the data obtained from measurements of renal cortical blood flow, determination of total renal flow rates failed to provide a reliable predictive index of organ function after retransplantation. Total renal flow rates of 22 cc/min or less occurred in 32% of kidneys exhibiting good cortical blood flow, while 20% of kidneys with poor cortical flow showed a total renal flow rate of 17 cc/min or more. In the seven dogs that underwent renal autotransplantation after scintigraphy, there was also no correlation between the total renal flow and posttransplant renal function. There was, however, an excellent correlation between renal cortical activity ratio and the function of the same kidney after retransplantation (Fig. 6).

DISCUSSION

It is generally agreed that ischemic insults to the kidney are associated with a marked decrease in blood flow to the outer renal cortex, and a concomitant increase in flow to the juxtamedullary glomeruli (*15*). Prolongation of the ischemic insult produces a further reduction in cortical blood flow and patchy perfusion of the cortex, eventually leading to acute tubular necrosis (*16*). In further studies of the effects of ischemia upon the microcirculation of the cold-preserved kidney by using the xenon washout technique, Corica et al. (*17*) observed significant decreases in blood flow to the outer cortex in kidneys exposed to 30 min of warm ischemia. Additional evidence of decreased blood flow to the outer cortex, with concomitant increases in blood flow to the juxtamedullary region, was provided in cold-preserved canine kidneys by Miller et al. (*6*), using microsphere injections. Here also, kidneys exposed to 30 min of warm ischemia failed to function after preservation and retransplantation into the injured host.

A number of other approaches to assessment of pre-transplant kidney viability have been reported, including Tc-DPTA scintigraphy (*18*) and renal angiography (*19*). These methods were generally of poor reliability, however, and angiography runs the additional risk of damaging the preserved kidney.

The potential usefulness of the microsphere injection technique in providing a reliable index of the status of the kidney has been enhanced considerably in recent years, particularly through the development of microspheres of precisely known diameter. This has been of particular value in studies of the renal microcirculation. It has been shown that injected radiolabeled microspheres measuring 15 μ in diameter are totally extracted from the circulation within a short time, and that under normal conditions, 99% of these microspheres become trapped in the glomeruli (*10*). Neither vasodilatation nor vasoconstriction of canine kidneys by pharmacological agents has produced skimming effects or a shunting of the injected microspheres from cortex to medulla (*10*). In addition, calculations of renal blood flow based on the microsphere injection technique have correlated closely with parallel results provided by electromagnetic flowmeters, and determinations of single-nephron glomerular blood flow by the microsphere technique have corresponded closely with the results obtained by micropuncture of the nephron (*10*). Trapping of injected microspheres in the glomeruli does not produce measurable effects upon glomerular filtration rates or sodium excretion (*10*). Relatively small doses of microspheres are required for performance of blood-flow studies, since less than 10% of the glomeruli need to be filled in order to provide statistically reproducible results (*20*). The safety of the technique is further enhanced through the use of biodegradable albumin microspheres, which are eliminated by macrophages within 3 hr after injection (*9*).

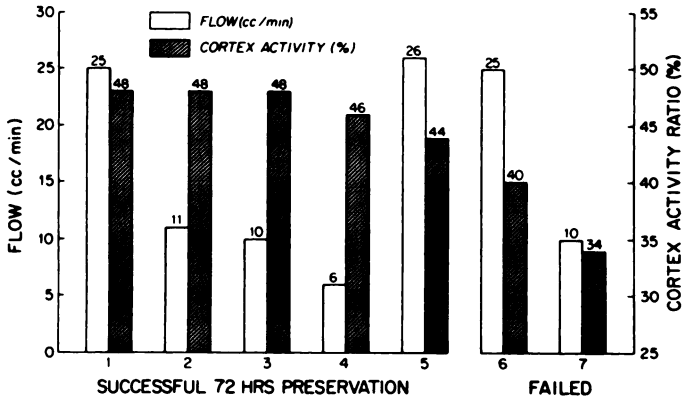

FIG. 6. Kidneys auto transplanted in 7 dogs after 72 hr of cold preservation: survival correlates with CAR, but not with total renal blood flow.

Studies of the renal microcirculation by the microsphere technique, however, have required preparation of sections of each tested kidney for gamma-counter analysis and/or autoradiography. Neither method permits transplantation of the kidney after these blood-flow determinations are completed. As a consequence, this valuable method has not thus far been applicable to the preoperative screening of preserved kidneys. The results of the present study suggest that scintigraphy can provide a reliable and reproducible index of the status of the renal microcirculation without interference with the subsequent transplantability of the tested kidney.

Previous studies from this laboratory indicate survival rates of 100%, 66%, and 33%, respectively, for canine kidneys flushed with Collins' solution and preserved in the cold for 24, 48, or 72 hr (*12*). Scintigraphic studies of kidneys treated in the same manner indicate that the decrease in kidney viability observed with increases in cold preservation time is associated with a progressive deterioration in the integrity of the renal cortical microcirculation. Addition of a calmodulin inhibitor (TFP) to the Collins' solution increased the survival rate of kidneys stored for 72 hr from 33% to 80%, and as shown by scintigraphic studies, also protected microcirculatory integrity in such kidneys (Fig. 7). Although there was an excellent correlation between maintenance of microcirculatory integrity (as determined by scintigram) and the ability of a particular kidney to sustain life after autotransplantation (Fig. 6), the data for total renal flow bore no evident relationship to either renal cortical flow or renal function after transplantation.

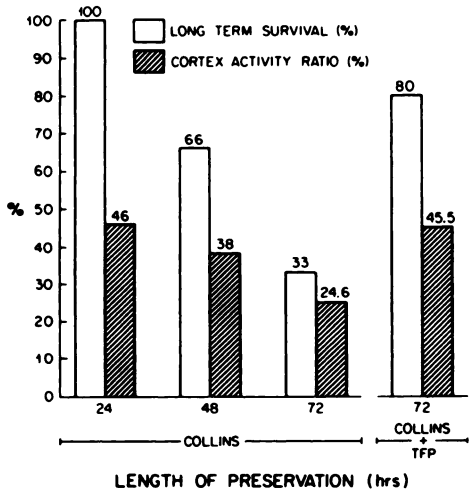

FIG. 7. CAR indicates chance of autotransplant survival after various periods of cold preservation.

Taken together, the results of this study suggest that the viability and function of a preserved kidney upon transplantation may be directly related to the degree of intactness of the renal cortical microcirculation. Gamma imaging of the renal cortical microcirculation has been shown to provide a reliable and safe noninvasive technique for assessment of kidney viability before transplantation. Utilization of this approach may be of value in the prevention of the thus far poorly predictable complication of acute renal tubular necrosis after transplantation from a cadaver donor to a recipient.

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