Kidney Storage in an Intracellular Type Solution

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The evaluation of a method used for renal preservation is dependent on the ability of the preserved kidney to support life immediately following implantation. The degree of renal function seen in the immediate post-transplant period has been employed as an index for the success of the preservation method (Collins et al, 1969; Hartley et al, 1971). Cortical ischaemia may be a principal cause for the renal damage as demonstrated by histological studies and expressed by renal functional impairment (Belzer et al, 1970). On the other hand, the long term, ie 6 to 12 months follow-up of the initial damage due to preservation can be further studied with benefit by evaluation of long-term studies of experimental renal autografts. Such studies have attracted little attention (Lempert & Blumenstock, 1971). It has been reported in experimental studies that kidneys stored in hypothermia and washed out with extracellular solution, developed advanced damage of glomeruli and tubules with a tendency to hypertension two years following storage and autotransplantation (Lempert & Blumenstock, 1971; Simso et al, 1963). After hyperbaric, hypothermic storage and autotransplantation the changes were similar but less pronounced (Lempert & Blumenstock, 1971). This may also be the case for kidneys stored in intracellular type electrolyte solutions. Histological changes have been observed in a majority of the kidneys stored in such an intracellular solution and reinserted thereafter (Collaborative study from four Paris Hospitals, 1972). Such changes could be the result of preservation ischaemic damage or a result of other unknown post-transplant complications and may be associated with the pathogenesis of hypertension after allografting stored kidneys.

In the present study we report on the status of renal function, the histology, the arterial blood pressure, and the peripheral blood levels of renin and erythropoietin in dogs, two years after renal storage in an intracellular type solution and autotransplantation.
MATERIALS AND METHODS

Five healthy adult mongrel male and female dogs weighing between 12 and 16 kg were used in this study. The animals were housed and observed in the Roswell Park Memorial Institute Experimental Surgery Department. After careful nephrectomy all kidneys were initially perfused with Ursol* solution and stored in a container with sufficient solution to cover the organ. The container was kept in a refrigerator for 24 hours at 4-8°C. Mannitol or phenoxybenzamine were not used in the study. The kidneys were then reinserted into the groin, and at the same time a contralateral nephrectomy was performed. The time between clamping the renal artery and the institution of chilled perfusion was referred to as warm ischaemia and did not consume more than ten minutes. The time of vascular anastomosis ranged between 20-30 minutes.

Blood urea nitrogen (BUN) and clearance of endogenous creatinine for glomerular filtration rate (GFR) were used for renal function evaluation. Blood samples for GFR, BUN, and haematocrit estimations were collected twice per month in the first three months and once every three months thereafter. Intravenous pyelography was also performed 3, 12 and 24 months following transplantation in each animal. Renal plasma flow was measured after a single injection of $^{131}$I-orthiodohippurate with sampling at five and fifteen minutes to determine the clearance of hippurate (Vityé & LeBel, 1969). Three to four estimations were carried out after the first three months. Plasma renin activity (PRA) was assayed using a radioimmunoassay technique and was expressed as nanograms of angiotensin I produced per hour per ml plasma (Haber et al, 1969). Plasma erythropoietin (ESF) was assayed by measurement of $^{59}$Fe uptake in polycythaemic mice (Mirand et al, 1959). Results were expressed as the ratio of the uptake of $^{59}$Fe in test samples divided by the percentage of $^{59}$Fe uptake in control animals injected with saline. Samples for hormone assay were collected every three months. Femoral blood pressure was measured by sphygmomanometer every two weeks.

An open renal biopsy was taken at the time of sacrifice or at one and two years after transplantation, for light and electron microscopic study. The juxtaglomerular index (JGI) of the afferent arteriole of the kidney was also evaluated, according to the scale of Hartroft and Hartroft (1953), in tissue specimens obtained by open biopsy.

RESULTS

Three animals are alive two years after autografting. The other two dogs.

*Ursol is the same as the solution C$^{3}$ of Collins et al (1969) with the difference that the magnesium sulphate concentration has been reduced by half to avoid the problems of precipitation, and the procaine concentration has been doubled (Varkarakis et al, 1973)
were sacrificed approximately 18 months post-operatively, following general anaesthesia for blood flow estimation. At that time their renal function and other measured parameters were stabilised compared with previous estimations.

Figure 1 illustrates the changes of GFR, BUN, systolic and diastolic blood pressure, plasma renin activity, and erythropoietin levels before and after renal preservation and reinsertion in all examined animals. This figure shows that renal function decreased three months post-operatively compared with pre-autografting values.

Systolic and diastolic blood pressures after two years differed little from pre-operative values. The small decrease in blood pressure at the end of the first year period was not significant \( p > 0.2 \). Similarly, PRA and ESF activity remained close to the pre-operative values during the experimental period \( p > 0.2 \). The haematocrit post-operative values (mean = 42.78 ±
1.98%) were not changed compared with pre-operative values (mean Hct = 40.2 ± 2.1%) (p > 0.5).

Intravenous pyelographic examination revealed normal excretory renal function without any obstruction in the urinary system in all animals. The renal plasma flow on all animals was stable between the third post-operative month and at the end of a two year period. These values ranged between 122-195 ml/min with a mean value of 156.42 ± 10.10 ml/min. The mean pre-operative value was 180 ± 15 ml/min. Light and electron microscopy studies showed normal histological appearances of the kidney. Particular attention was paid to the renal vessels for hypertensive changes, or renal tubules for ischaemic abnormalities. The JGI in the specimens examined (mean value = 14.5 ± 2.3) was not altered significantly compared with the value in seven other normal biopsied dogs (mean value = 13.1 ± 2.7) (p > 0.5).

DISCUSSION

The flush-out and storage of kidneys with intracellular type electrolyte solution is a simple and inexpensive method which has been used successfully for renal transplantation, experimental or clinical, with cadaveric donors (Collaborative study from four Paris Hospitals, 1972; Collins et al, 1969). In the present study the animals exhibited normal renal function. Renal blood flow was stabilised following the three months post-operative period, with a modest and transitory 15% decrease from pre-operative values.

Peripheral levels of erythropoietin were the same before and after renal preservation. This suggests that significant intra-renal ischaemia or hypoxia had not occurred (Jacobson et al, 1957). It is known that the erythropoietic hormone is produced or activated in the kidney (Jacobson et al, 1957; Murphy et al, 1968), in fact the dog does not have an extra-renal source of ESF (Mirand et al, 1968). We concluded that the ischaemic trauma of renal preservation and denervation did not affect the elaboration of erythropoietin in this study. The normal haematocrit levels support this view.

In the present study the animals were not hypertensive during prolonged follow-up, a finding which seems to have special value in view of previous reports stating that simple hypothermia appeared to induce hypertension in experimental animals (Lempert & Blumenstock, 1971; Simso et al, 1963). The normal blood pressures observed in this series are supported by the various determinations performed including normal values of plasma renin activity, normal granularity of the afferent arteriole of the juxta-glomerular apparatus, and lack of hypertensive arterial lesions on light and electron microscopic examinations. These results suggest that the histological changes reported elsewhere after renal transplantation in kidneys stored in intracellular solution (Collaborative study from four Paris Hospitals, 1972) may not be related to the ischaemic injury of the preservation method.
In our study the warm ischaemia time was very short. It had been shown previously that when warm ischaemia is longer than 15 minutes, successful renal preservation with intracellular solution is not possible (Frost et al, 1970; Scott et al, 1971). This finding is a practical consideration in the clinical use of cadaveric kidneys with the development of increased vascular resistance so frequently noted in the agonal phase of death (Belzer et al, 1970). However, it has been shown that after one hour warm ischaemic time with the use of C2 Collins' solution containing heparin and chlorpromazine, a successful 24 hour preservation of pig kidney was achieved (Løkkegaard et al, 1971). Similar results were reported in cadaver kidneys having one hour warm ischaemia and stored for 12 hours in intracellular solution (Collaborative study from four Paris Hospitals, 1972).

In conclusion, the results show good renal function, histological integrity, normal hormonal release, and normal blood pressure in animals receiving autotransplanted kidneys stored by a simple technique. In the absence of similar data for kidneys preserved with continuous perfusion, these results favour the employment of this relatively simple storage method.

SUMMARY

Kidneys preserved in intracellular solution functioned well two years after reinsertion. Normal renin and erythropoietin levels were associated with normal blood pressure, and haematocrit. Hypertensive changes reported in clinically allografted kidneys preserved with the same solution are, therefore, possibly related to other post-transplantation allograft complications. This simple technique of renal storage thus gives evidence of subsequent good renal function as determined during prolonged evaluation period.

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OPEN DISCUSSION

P J C VEREERSTRAETEN (Chairman): It has been stated that arterial hypertension is frequently observed after autotransplantation in kidneys stored without perfusion, but that this hypertension disappears if the survival time of the graft survives the six month period. Is this statement consistent with the observations of Drs Varkarakis and Murphy?

S SHALDON (London): I think that the paper as I read it would suggest that there was alteration in the first three months, but that subsequently everything appeared to be normal. Without the data this question is difficult to answer, but my impression is that they get changes in the first three months, and then things stabilise. I do not think that one can be dogmatic, but I feel that the authors have demonstrated that you can perform a renal autotransplant after a long period of preservation and they have confirmed what was known already.