Successful Hypothermic Kidney Storage for 24 Hours Using an Intracellular Type of Perfusate

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It is well known that most efficient use of cadaver tissues in transplantation is facilitated by short term organ preservation. This allows time for histocompatibility testing and eliminates much of the haste and urgency in transplant operations.

Various preservation techniques extend the tolerable ischaemic period of organs to be used in transplantation. These techniques range from simple immersion cooling to complex continuous perfusion methods using blood or blood-component perfusates.

Until recently, the best results were obtained using continuous perfusion with hypothermia which allowed for successful 24 hour preservation of dog kidneys in the great proportion of experiments. An introduction of a new 'intracellular' type of solution by Collins (Collins et al, 1969) drew attention to the possibility of simple hypothermic kidney storage without a need for complex and expensive perfusion machines. There is, however, some controversy about the results obtained by various investigators using this solution in dog kidney preservation. In most experiments the kidney to be preserved is taken out from a living animal with great operative care and usually reimplanted to the same animal after storage. The present work was undertaken to compare results of 24 hour preservation of the dog kidney taken from living and simulated cadaveric donors.

MATERIAL AND METHODS

Experiments were performed on 31 mongrel dogs in two groups. Group I consisted of 20 dogs when reimplantation of the preserved kidney was done, and Group II consisted of 11 dogs which received allotransplants of cadaveric preserved kidneys.

Group I

Dogs were anaesthetised with eunarcone. Special care was taken to avoid
hypotension during induction of anaesthesia and during the surgical procedures. Dogs were kept well hydrated receiving 1000 ml of normal saline and 5% dextrose intravenously. Through a midline incision the left kidney was dissected with special care, avoiding unnecessary handling, and taken out. Immediately after excision the kidney was perfused using hydrostatic pressure with 60 - 100 ml of Collins' solution. Perfusion flow was measured. The perfusion was stopped when the effluent was clear. The kidney was placed in a sterile flask filled with the same solution and kept in a refrigerator (+4°C) for 24 hours.

After 24 hours storage the renal vessels were anastomosed to the iliac vessels of the donor animal and a ureteroneocystostomy was done. Before revascularisation contralateral nephrectomy was done. During operation the dogs were given normal saline and 5g/100ml dextrose. After revascularisation of the kidney dogs were given 7.5 g of mannitol.

During the first two days the dogs were given iv. or s.c. infusions and food and water ad libitum thereafter. Serum creatinine and blood urea were determined daily for 10 - 14 days.

Group II
Donor dogs were anaesthetised with eunarcone and haemorrhagic hypotension was produced by sudden arterial bleeding (on average 35 ml/kg body weight) to a mean arterial pressure of 40 - 60 mm Hg. At the same time dogs were infused with 0.9 g/100ml saline containing 25 mg of phenoxybenzamine. Dogs were kept hypotensive for 15 minutes and then killed with iv. injection of 10 g/100ml potassium chloride. Both kidneys were taken out and then perfused as in Group I with cold (+4°C) Collins perfusate and placed in the same solution in a refrigerator. Warm ischaemia time in these kidneys ranged from 20 - 30 minutes.

After 24 hours storage both kidneys were transplanted into bilaterally nephrectomised dogs. Surgical procedure and the post-operative care did not differ from those in Group I. No immunosuppression was attempted.

Viability assay
In both groups metabolic activity of the kidney was measured using tetrazolium bromide (TTB) viability assay. The test consists of placing a solution of tetrazolium salt on a 1 mm³ section of tissue. The presence of a functionally intact enzymatic complex for hydrogen-ion transport causes a reduction of the salt to coloured water-insoluble formazan. The time, in seconds, which elapse before the appearance of the first perceptible darkening of the tested tissue is considered to be proportional to the function of dehydrogenase enzyme systems and thus to the viability of the organ (Terasaki et al, 1967). TTB viability assay was done twice in each experiment - after exposure of the donor kidney and after revascularisation of the preserved kidney.
RESULTS

Group I

The perfusion flow in these kidneys ranged from 12 to 25 ml/min. The time of formazan production (TTB viability assay end point) before preservation varied from 18 to 21 sec and after revascularisation of stored kidney rose to 24 seconds (20 - 25 seconds). In all animals the kidney excreted urine shortly after revascularisation.

In 8 animals postoperative kidney function was good with a maximal rise of serum creatinine to 2.1 mg/100ml: 8 dogs developed reversible uraemia (maximum serum creatinine 6 mg/100ml) but in 4 of them there were some postoperative complications which might have influenced kidney function (intestinal obstruction in 3 and hydronephrosis in 1). Four dogs died early in the postoperative period – two due to technical faults, and two others from uraemia.

Microscopic examination of kidneys taken from two dogs which died of uraemia showed tubular necrosis. In one dog which died with good kidney function 6 months after transplantation due to distemper microscopy revealed practically normal appearance of all kidney structures.

Group II

The perfusion flow before storage varied from 6 to 15 ml/min. TTB end point time was measured only after revascularisation and ranged from 30 to 35 seconds. In all but one of the dogs the kidneys excreted urine some time after revascularisation. All dogs were uraemic. Survival time ranged from 4 to 15 days. An autopsy done in all dogs revealed viable but grossly rejected kidneys. In 5 animals which died on the 4th, 5th and the 6th postoperative days an intussusception was also found.

Microscopical examination revealed viable kidneys with moderate to severe tubular necrosis and the classical findings of rejection (mononuclear cell infiltrates and fibrinoid necrosis of the vessels).

DISCUSSION

In human kidney transplantation there is as yet no real need for 24 hours storage as current histocompatibility tests can easily be performed in 8 - 12 hours. Simple hypothermic 24 hours storage of the kidney using an intracellular type of solution was successful in most (90%) animals in our experiments. TTB end point time was short and did not differ much from controls.

Unlike the organs removed from living donors cadaveric organs are exposed to far greater metabolic hazard. This is due not only to anoxia following death, but also to poor perfusion during the period of clinical deterioration prior to death. The second group of experiments was done to
simulate this clinical situation. Although all dogs were ureaemic, all but one kidney excreted urine. TTB end point was prolonged indicating disturbance of tubular enzymatic systems. Although microscopical examination of the kidneys showed the typical pattern of tubular necrosis the kidneys were viable. Typical rejection pictures were also found in these kidneys.

Collins' solution was recently used in two human cadaveric kidney transplantations in Warsaw Centre. In the first case the kidney after 150 minutes of total ischaemia excreted urine immediately after revascularisation. Unfortunately a hyperacute rejection occurred 1 hour after transplantation and the kidney had to be removed. In the second case total ischaemia time was 4 hours and the patient underwent a period of oliguric acute tubular necrosis, confirmed by biopsy of the kidney.

REFERENCES
Terasaki, P. I., Martin, D. C. and Smith, R. B. (1967) Transplantation, 5, 76

OPEN DISCUSSION

W J KOLFF (Salt Lake City, Chairman): Thank you very much Dr Rowinski, I would like to compliment you on your extremely attractive slides. All your dogs have their tails up! All your slides can be read – I have seen many slides here that I am sure were made by professional artists and which I have not been able to read.

J DORMONT (Paris): Have you done an autograft series like your series number two with warm ischaemia of say half an hour at 37°C followed by re-implantation into the original animal? This is a very interesting problem because most studies with Collins' fluid have been done without a warm ischaemic time and the results are certainly good. The question you have asked is a very interesting one, but unfortunately the rejection process made the thing very difficult to interpret.

ROWINSKI: We thought about this and intend to do it but it is quite difficult to have a warm ischaemia of this type. We tried to simulate the clinical situation with a period of poor perfusion but these dogs with haemorrhagic hypotension were still alive and this is a bit different from the situation where you take a kidney out and put it on the table for half an hour and then transplant it back. You would have to clamp the renal arteries and give
heparin because everything would clot inside the kidney. For this reason we chose this particular experimental protocol but I quite agree with you that it would be much better to have the set-up which you mention.

BILDE (Copenhagen): We have done similar experiments in pigs to those you have done in dogs. We pre-treated the pigs with chlorpromazine and heparin and then ligated the vessels for one hour – that means one hour of warm ischaemia. We had two groups of five pigs with one group as controls. Four control pigs died in uraemia and one survived. In the chlorpromazine and heparin group all the pigs survived and lived for one month before they were killed. At this time the serum creatinine had been reduced by 10%. I cannot say any more about this because the work is still going on.

KOLFF: The problem is that the usual cadaver donor killed in an automobile accident is not usually given heparin and chlorpromazine immediately!