Hyperbaric Hypothermic Perfusion
Preservation of Ischaemic Kidneys
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Kidneys used in clinical renal transplant programmes in Britain are frequently exposed to a warm ischaemia interval of over 30 minutes. This insult usually produces tubular necrosis with anuria in the immediate post-operative period making dialysis necessary and rejection episodes difficult to diagnose. Experimental assessment of organ preservation techniques is usually performed on organs with no warm ischaemia (Brunius et al, 1968; Ladaga et al, 1968; Hitchcock et al, 1964; Manax et al, 1966) often after pretreatment of the donor by a variety of drugs (heparin, phenoxybenzamine, chlorpromazine) or by hydration. This type of experiment has led to the widespread use of particular solutions in clinical practice (Brunius et al, 1968; Collins et al, 1969). Unfortunately it is not always possible to pretreat human kidney donors and rarely possible to avoid warm ischaemia. The purpose of this study was to assess several preservation methods using the dog kidney as a model, in which the kidney was exposed to a standard 40 minute warm ischaemia interval without any form of pretreatment of the donor.

MATERIALS AND METHODS

Mongrel dogs weighing 15 - 28 Kg were starved for 18 hours before the experiment. Anaesthesia was induced with thiopentone and maintained via an endotracheal tube with an oxygen, nitrous oxide halothane mixture. Isotonic saline solution (20 ml/Kg) was infused intravenously over 30 minutes and the right kidney was mobilised through a midline incision. Following division of the ureter, a soft clamp was applied to the renal vessels which were then divided and the kidney was quickly transferred to a sterile polythene bag which was immersed in a water bath at 37°C. After 40 minutes, the kidney was removed and allocated to one of the preservation methods.

Group 1 - Ice storage alone
The kidney was surrounded by ice and stored at a temperature of 2-4°C without perfusion.
Group 2 - Brunius and Gelín technique (Brunius et al, 1968)

The renal artery was cannulated and 20 ml of 1/2% lignocaine with 5,000 U of heparin was injected into the renal artery. This was followed by infusion with 1 ml/g of 'Perfudex' solution (Pharmacia) at a pressure of 100 cms of water. Lastly a solution (1 ml/g) containing equal volumes of 10% Fructose and 1.4% NaHCO₃ was infused at 4°C and a pressure of 100 cms of water. The kidney was then surrounded by ice and stored at 4°C.

Group 3 - Modified Dextran 40 solution

The kidney was perfused with a 5% Dextran 40 solution (1.5 ml/g) at a pressure of 100 cms of water. The electrolyte concentrations of this solution were sodium 140 mEq/l, potassium 2.8 mEq/l and chloride 150 mEq/l. The final solution closely resembled 'Perfudex' (Pharmacia) except that it did not contain magnesium.

In the remaining three groups the kidneys were preserved by continuous machine perfusion using a Vickers Hyperbaric perfusion apparatus (Figure 1). In each of these three groups the renal artery was injected with 2.5 mg of phenoxybenzamine in 0.5 ml saline immediately after nephrectomy. Thereafter, the organ was infused with a solution based on that used by Snell and Hopkinson (1972) at 4°C and 100 cm of water for 5 minutes. The same

Figure 1

480
solution was later used to perfuse the kidney at 2 ml/minute using a gas driven pump with a filter and a non-circulating system. The solution had an intracellular electrolyte composition (Collins et al, 1969) modified by the addition of 5% Dextran 40 and was made up by adding equal volumes of the electrolyte solution and rheomacrodex in 5% dextrose. Heparin (5,000 units), procaine (10 ml of 1%) and chlorpromazine (16 mg) were added to 500 ml of the perfusate immediately before use. Groups 4, 5 and 6 kidneys were all perfused by this solution but differed in the content or timing of addition of phenoxybenzamine. In Group 4, phenoxybenzamine (25 mg) was added to the cold electrolyte solution 48 hours before use and then stored at 4°C. In Group 5, phenoxybenzamine (25 mg) was added to the solution immediately before use and in Group 6 phenoxybenzamine was not used at all.

Kidneys in Groups 1, 2 and 3 were stored for 6 hours in ice at 4°C. Kidneys in Groups 4, 5 and 6 were stored in a Vickers portable renal preservation unit (Hopkinson & Snell, 1972) shown in Figure 1. The organs were perfused at 2ml/min for 4 hours at 5°C and 3 atmospheres of oxygen. Hyperbaria and hypothermia were then maintained for a further 20 hours.

After storage, the donor animals were re-anaesthetised and the kidney re-implanted into the left iliac fossa by end to end anastomosis between the renal artery and external iliac artery and end to side anastomosis between the renal vein and common iliac vein. The ureter was implanted directly into the bladder. Before revascularisation in Groups 4 - 6, 2.5 mg of phenoxybenzamine in 0.5 ml of saline was injected into the renal artery.

The mean revascularisation time was 29 minutes. Following implantation, mannitol (10%, 4 ml/Kg) was given to the animal in addition to 1 litre of isotonic saline. Contralateral nephrectomy was performed immediately after revascularisation.

After operation a further litre of isotonic saline was infused and the dogs were permitted to drink at will. Food was allowed on the second day. If post-operative ileus occurred the animals were given further intravenous or subcutaneous infusions of normal saline until alimentation returned to normal. Serum creatinine and blood urea levels were measured twice weekly until death or sacrifice after which the kidneys were submitted to pathological examination.

RESULTS

Animals dying from renal artery thrombosis or intussusception were excluded from analysis.

In Group 1, 1 out of 7 animals survived. In Group 2, 2 out of 6 animals survived and in Group 3, 3 out of 10 survived. Mortality in all groups was due to acute tubular necrosis. The survivors had poor initial renal function which took an average of 18 days to return to normal. The mean maximum

481
serum creatinine was 7.4 mg% (Table I) and the mean maximum blood urea was 362 mg%.

<table>
<thead>
<tr>
<th>Preservation system</th>
<th>Number of animals</th>
<th>Survivors</th>
<th>Peak serum creatinine of survivors (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Infusion</td>
<td>7</td>
<td>1</td>
<td>8.3</td>
</tr>
<tr>
<td>Brunius &amp; Gelin</td>
<td>6</td>
<td>2</td>
<td>7.0, 6.4</td>
</tr>
<tr>
<td>Perfudex</td>
<td>10</td>
<td>3</td>
<td>10.2, 5.8, 7.1</td>
</tr>
</tbody>
</table>

Kidneys were exposed to 40 minutes of warm ischaemia and 6 hours of cold ice storage.

In Group 4, 5 out of 6 animals survived with a mean maximum blood urea of 243 mg% and mean serum creatinine of 4.7 mg%. All 5 had returned to normal values by the fourteenth day. The kidney of the sixth animal showed the histological features of acute tubular necrosis. In Group 5, there were no survivors and all organs showed acute tubular necrosis with areas of patchy cortical necrosis. In the last group (6) 2 out of 6 animals survived, the remainder again dying of acute tubular necrosis. These results are summarised in Table II.

<table>
<thead>
<tr>
<th>Hyperbaric hypothermic perfusion</th>
<th>Number of animals</th>
<th>Survivors</th>
<th>Mean peak serum creatinine of survivors mg%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenoxybenzamine (clear solution)</td>
<td>6</td>
<td>5</td>
<td>3.3, 4.3, 4.5, 5.4, 6.0</td>
</tr>
<tr>
<td>Phenoxybenzamine (cloudy solution)</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No Phenoxybenzamine</td>
<td>6</td>
<td>2</td>
<td>4.4, 6.3</td>
</tr>
</tbody>
</table>

After 40 minutes of warm ischaemia, the kidneys were perfused for 4 hours and then stored for a further 20 hours under hypothermic, hyperbaric conditions without perfusion.

DISCUSSION

Although a number of solutions have been advocated for clinical organ preservation, these solutions are often based upon experimental work in which kidneys are not exposed to a warm ischaemia interval and the donor is usually pretreated in a variety of ways. Unfortunately, in clinical practice organs are frequently exposed to a warm ischaemia for more than 30 minutes without donor pretreatment. The object of this study was to try
to mimic the clinical situation by attempting to obtain immediate renal function after significant warm ischaemia without donor pretreatment.

The duration of warm ischaemia tolerated by the dog kidney was assessed by Sells and Pena (1970), who found that 40 minutes of warm and 5 hours of cold ischaemia resulted in very few survivors. We used 40 minutes of warm and 6 hours of cold ischaemia and attempted to produce immediate renal function by the use of a number of different but accepted preservation methods. Minor reductions in body temperature could conceivably have protected organs left free in the peritoneal cavity. The period of warm ischaemia was therefore standardised by maintaining each kidney at a temperature of 37°C in a water bath. In addition, decompression of the kidney was prevented by applying a clamp to the renal vessels before division.

In the first group of experiments in which the kidneys were simply stored in ice after the period of warm ischaemia, only two animals survived with immediate renal function. These figures were not improved by infusion with 'Perfudex' or after treatment by the solutions recommended by Brunius and Gelin (Brunius et al, 1968) before storage in ice. The survivors in all groups had poor renal function taking an average of 18 days to return to normal.

In order to try and achieve immediate renal function we decided to assess the potential of continuous perfusion in this model. Belzer et al (1967) and Johnson et al (1972) have shown that by using continuous perfusion with lipoprotein free plasma or albumin, immediate function after warm ischaemia can be achieved. We chose instead to assess the Vickers hyperbaric hypothermic preservation unit using a modified Collins' solution for the perfusate (Snell & Hopkinson, 1972). This method had the potential advantages that the machine is portable and a readily available electrolyte solution could be used as the perfusate. Using this technique with four hours perfusion and 20 hours of cold ischaemia 5 out of 6 organs survived with immediate life sustaining function. Renal function was excellent, the maximum mean creatinine reaching 4.7 mg%, and normal renal function returned within 14 days. In these experiments phenoxybenzamine had been added to the electrolyte solution 48 hours before use, since after addition at 4°C the solution becomes cloudy and takes 48 hours to clear. In order to assess the importance of this step, two other groups of animals were investigated again using machine perfusion. The first set of kidneys was perfused with the cloudy solution resulting from addition of phenoxybenzamine, but none of these animals survived. In the second group, phenoxybenzamine was not added to the solution and only 2 of 6 animals survived. Consequently, phenoxybenzamine is essential in this system and the solution has to be clear.

The addition of phenoxybenzamine to a solution 48 hours before use poses a number of logistic problems in anticipating donors. These can
however be overcome if phenoxybenzamine is added to the solution at room temperature, when the solution clears within 4 hours.

Hyperbaria seems to be important. Hopkinson (1971) has shown that if hyperbaria is omitted, the results of preservation are inferior. However, the mechanism of action of hyperbaria remains to be elucidated.

From these results it is clear that further damage may be produced by some methods of washout preservation when kidneys have been damaged by warm ischaemia. Immediate function after transplantation is a desirable aim as it abolishes the dangers of post-operative dialysis and makes the early detection of rejection much simpler.

The Vickers machine is completely portable and can be taken to the prospective donor obviating the need for a period of non-perfusion while the organ is transported to the machine.

SUMMARY

Kidneys were removed from groups of dogs and exposed to 40 minutes of warm ischaemia and 6 hours of cold ischaemia before re-transplantation and immediate contralateral nephrectomy. In the first group the organs were merely stored in ice and 1 out of 7 animals survived. In Group 2 the organs were infused with the solutions suggested by Brunius and Gelin (Brunius et al, 1968) after the warm ischaemia interval and then stored in ice. In this group, 2 out of 6 animals survived. In the third group, the organ was infused with a modified 'Perfudex' solution with 3 out of 10 survivors. Three further groups were investigated using hypothermic hyperbaric perfusion for 4 hours following warm ischaemia and 20 hours of hyperbaria without perfusion. An electrolyte solution based on Collins' solution was used as the perfusate. In the first of these groups phenoxybenzamine was added to the solution 48 hours before use with 5 out of 6 survivors. In the second group phenoxybenzamine was added immediately before perfusion, the resulting cloudy solution being utilised. There were no survivors in this group. In the last experiment phenoxybenzamine was omitted and only 2 out of 6 animals survived.

REFERENCES

Hopkinson, W. I. and Snell, M. E. (1972) Proceedings of the 4th International Course on Transplantation, Lyon

484
OPEN DISCUSSION

S KADJEI (Edinburgh): You mentioned that the phenoxybenzamine has to be added 48 hours before use. Assuming that you do not know when a kidney is going to become available, is it a good idea to make a clear solution, add the phenoxybenzamine and keep it for more than 48 hours? How do you overcome the 48 hour deadline?

QUIN: The deadline does of course cause a problem, but since commencing this experiment we have overcome it by adding the phenoxybenzamine to the solution at 4°C. This procedure takes two days.

H KREIS (Paris): If you want to prove that kidneys preserved on the Vickers' machine do better than those that have been preserved on washout you have to use the same kind of solution, ie Perfudex. As you know, we are able to preserve kidneys for 48 hours with a 45 minute warm ischaemia time in Collins’ solution.

QUIN: I am aware of this fact, and I also know that in at least three papers that have been published, where warm ischaemic intervals of 50 to 60 minutes have been used, and dog kidneys have been perfused with Collins' solution, the kidneys have not worked.

J HOELTZENBEIN (Münster): Is there a decompression period when the kidneys are taken out of the hyperbaric machine?

QUIN: We simply place a soft clamp across the renal artery, divide the vessels, take it straight out and place it in a sterile bag. Is this what you mean?

HOELTZENBEIN: I am not sure that I understood: I was, in fact referring to the hyperbaric decompression period.

QUIN: The decompression is carried out over a period of about half an hour.
W ROWINSKI (Warsaw): Do you have any idea what causes the cloudiness to which you refer? I do not understand why when you add the phenoxybenzamine 14 hours earlier it works, and if you add it 10 minutes earlier it does not. I also do not believe that phenoxybenzamine works at $4^\circ$C. My comment is that we have been using Collins' solution for experimental and clinical kidney preservation, and we have done some experiments with 30 minutes warm ischaemia and 24 hours cold ischaemia. Our kidneys worked.

QUIN: I do not know why they worked either, but they did and we did not use phenoxybenzamine at all. As far as the cloudy solution is concerned I think that on looking at the histology of the kidneys you see many microemboli. There is a severe vascular problem, presumably from crystals in the solution.

As far as your second question is concerned, I agree that with 30 minutes warm ischaemia survival occurs as you describe. We use 40 minutes which is approaching the maximum. I think that 30 minutes results in less injury, and I think it is important to ensure that warm ischaemia time is controlled, because if you take the kidney out or leave it inside the dog it has been shown that the temperature can fall by 3 or 4 degrees centigrade over a period of about half an hour.

ROWINSKI: I quite agree: we used to clamp the renal artery, leave the kidney in the body and close the abdomen. The temperature, therefore, had to be between 35 and $34^\circ$C. How much magnesium are you using in your Collins' solution? The crystals in your solution are probably due to too much magnesium, not phenoxybenzamine.

QUIN: You may well be right: I am not sure why these crystals occur, but in this situation those are the results.