EXPERIMENTAL NORMOTHERMIC KIDNEY PRESERVATION: HISTOLOGICAL CONSIDERATIONS

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Introduction

Despite much clinical and experimental work carried out over the past fifteen years in the field of hypothermic kidney preservation, 72 hours remains the maximum clinically useful preservation period. A new approach is needed to better utilise cadaver kidneys and allow for more elaborate testing and sharing.

Prior to the advent of perfluorochemical emulsions, in vitro normothermic renal perfusions lasting longer than four hours have been impossible to achieve; microembolism of lipid and red cell fragments occurred in kidneys perfused with suspensions of erythrocytes, destroying the renal microvasculature. Research by Clark [1], Geyer [2], Sloviter [3], and other has shown that perfluorochemicals can substitute for the red cell in its O₂,CO₂ transport function. This paper describes experiments in normothermic kidney perfusion using a fluorocarbon medium in place of an erythrocyte suspension.

Method

Nine canine kidneys excised during maximum diuresis were immediately placed in a special perfusion apparatus consisting of a plexiglass chamber, roller pumps, heat exchanger and bubble oxygenator (Figure 1). The flow rate was adjusted to 5cc/gm kidney, gassed with a 95% O₂, 5% CO₂ mixture and maintained at 37°C.

The kidneys were randomly assigned into two groups whose perfusates differed only in the presence or absence of perfluorotributylamine (FC-43). Each perfusate volume of 1455cc consisted of Pluronic F-68 2.56gm%, hydroxyethyl starch 3.0gm%, NaCl 95.8mEq/L, K Cl 5.2mEq/L, Ca Cl₂ 2.5mEq/L, Mg Cl₂ 1.47mEq/L, Na HCO₃ 35mEq/L, glucose 100mg %, heat inactivated calf serum 280ml, human albumin (25gm%) 100ml, intralipid (10% emulsion) 15ml, glutamine 500mg, protamine 250mg, cortisone acetate 20mg, insulin (reg) 4u, ephedrine 4mg, thyroxine sodium 100μg and Cephamandol 42mg.

Arterial and venous perfusate samples and needle biopsies were obtained initially and at 1, 3, 6, 12, 18 and 24 hours. Arterial and venous pH, PCO₂, HCO₃, total
CO₂ and base excess were determined using an ABL 2 Blood Gas Analyser. The O₂ consumption was calculated from the A-V O₂ content difference. The perfusate was also analysed for sodium, potassium chloride, CO₂, BUN, creatinine, glucose, calcium, magnesium, phosphorous, albumin, and lactic dehydrogenase levels at each time interval.

The needle biopsies were placed immediately in 0.1 M phosphate buffer/2.5 glutaraldehyde fixative (pH-7.30) for light and electron microscopic sectioning. Specimens were post fixed in 1% osmium/0.1 M phosphate buffer, and embedded in SPURR low viscosity plastic. One micron sections were cut, stained with methylene blue/azure 2, and examined by light microscopy. Two biopsies were excluded because cortical elements were not present. (A medullary biopsy had been obtained.) The tubular epithelium was evaluated regarding the brush border, degree of cytoplasmic vacuolisation, and patency of tubular lumens. Additional sections, 600–800 Ångstroms thick, from FC-43 and non FC-43 kidneys were obtained and examined by electron microscopy.

A perfusion was terminated when the pressure reached 220mmHg or when extravasation of perfusate from the cortical biopsy sites reduced the renal vein effluent sufficiently to preclude adequate sampling.
Results

All kidneys were perfused for 18 hours with the exception of two FC-43 kidneys that lasted 24 hours. The onset of visible swelling, which occurred in every kidney during the final 3–5 hours of perfusion, correlated with the onset of a rise in the perfusion pressure. Mean weight increases were 56 ± 5.3% in the FC-43 group (n = 5) and 51.9 ± 4.1% in the non FC-43 group (n = 4).

During the perfusions, the concentrations of sodium, potassium, chloride, CO₂, BUN, creatinine, glucose, calcium, magnesium, phosphorous, and albumin did not change in either group; however, all of the non FC-43 perfused kidneys released significantly greater amounts of LDH than FC-43 perfused kidneys. Similarly, the A-V O₂ differences were greater in the non FC-43 perfused kidneys.

Interestingly, O₂ consumption calculated from the perfusate flow rate, A-V O₂ difference, and the respective O₂ contents revealed no differences between the FC-43 and non FC-43 groups. Their rates of 3.5–5.23 vol%/100gm/min are less than that found in the in vivo kidney.

After equilibration with the Carbogen gas mixture, the mean pCO₂ value for the FC-43 group was 32.7 ± 0.7mmHg and for the non FC-43 group 35.27 ± 0.63 mmHg. However, the pCO₂ values were not significantly different although the pH of both groups fell after perfusion began. This drop in pH was more sustained in the non FC-43 group.

Figure 2. Light microscopy, methylene blue/azure 2 stain, 440X (reduced for publications). (a) the non FC-43 perfused kidney at 12 hours preservation. Note the swelling, occlusion of tubular lumens and cellular necrosis. (b) the FC-43 perfused kidney. Note the patency of the tubular lumens, integrity of the cells, and the intact brush border
The light and electron microscopy revealed significant differences between the two groups. In the methylene blue sections all the glomeruli were intact in both FC-43 and non FC-43 groups. The major changes affected the tubules, and two patterns emerged: 1) the non FC-43 group showed early loss of tubular brush border after one hour, cellular necrosis by three hours, and occlusion of the majority of the tubular lumens after 12 hours of perfusion (Figure 2a); 2) in the FC-43 perfused kidneys the brush border was well preserved and occlusion of the tubular lumens was rare. Nevertheless, a progressive cytoplasmic vacuolation was seen in the sections from the FC-43 kidneys taken at 12 (Figure 2b) and 24 hours of perfusion. The vacuoles showed no fluorescence under ultraviolet light nor the characteristic microscopic appearance of fluorocarbon particles.

Electron microscopic sections were performed at the 3 and 12 hour intervals for a non FC-43 and a FC-43 perfused kidney and at 24 hours for the FC-43 kidney. In the non FC-43 kidney, severe mitochondrial damage occurred as evidenced by swelling, loss of cristae and appearance of electron dense precipitants at 3 hours of preservation. Further, after 12 hours of perfusion complete loss of cellular integrity was seen (Figure 3a). In contrast, although the FC-43 kidney exhibited some mitochondrial swelling, the internal cellular architecture was well maintained in spite of the progressive cytoplasmic vacuolation described above (Figure 3b). At 24 hours, the mitochondria of the FC-43 preserved kidneys exhibited pleomorphism and swelling but remained intact (Figure 4).

Figure 3. Electron microscopy 27,500X (reduced for publication). (a) The non FC-43 perfused kidney at 12 hours. Note the cellular and mitochondrial degeneration. (b) The FC-43 perfused kidney at 12 hours. Note the intact organelles and the large vacuoles
Discussion/Conclusion

Normothermic perfusion preservation is possible using fluorochemicals for O₂-CO₂ transport. Although the LDH release, perfusate pH and the histology of the FC-43 group is superior to the non FC-43 group, progressive swelling prevented normothermic preservations longer than 24 hours. Since the O₂ consumption of the in vivo kidney is 5–7ml O₂/100gm/min, and since the FC-43 perfusate in these experiments delivers 17.9ml O₂/100gm/min, it seems likely from the physiological results that the deterioration in the FC-43 kidneys may be less related to hypoxia than in the non FC-43 group [4].

The light and electron microscopical findings correlate with the previously described histology. In the non fluorocarbon group the tubular necrosis, seen as early as 3 hours, seems likely to be caused by a relative tissue hypoxia, whereas the preservation of the brush border and mitochondria of the FC-43 group suggests adequate tissue oxygenation. The cytoplasmic vacuolisation of the FC-43 group may be caused by the fluorocarbon, the hydroxyethyl starch, the Pluronic F-68, and/or inadequate substrate/hormone homeostasis. However, the vacuoles themselves are not fluorocarbon, since they do not fluoresce with ultraviolet light and the characteristic histology of fluorocarbon particles was absent.

Interestingly, Thompson [5] has shown that hydroxyethyl starch can produce cytoplasmic vacuolisation and Roze [6] has demonstrated similar changes involv-
ing rat kidneys following administration of Pluronic F-68.

These preliminary experiments suggest further studies which should evaluate factors such as perfusate toxicity, substrate deficiencies, oxygen toxicity, or altered haemodynamics. Reimplantation studies at a variety of preservation times are a logical next step in evaluating the clinical potential of fluorocarbons for kidney preservation.

Acknowledgments

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References

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Open Discussion

KULATILAKE (London) I am sure you are aware of the work of Professor Nizet in Liège over a long period of years and my own work in London. In normothermic perfusion with dog kidneys, the secret we found was that the circulation time with whole blood has to be less than five minutes. If you keep to that you can perfuse a kidney for any length of time. My limitation was haemolysis of the blood, not the vasoconstriction. I perfused for fourteen hours. I did not transplant them as they were infected. The gentleman who is perfusing kidneys with defibrinated blood is wasting his time. Defibrinated blood is poison to the kidney tissue and I think you are on the right path, but may I suggest that you combine this with some of the normal substances you find in the blood.

MERKEL Thank you for your comments. This in effect is what we are trying to do. We are trying to add all the normal constituents to the perfusate that would normally be in the blood, i.e. hormones and nutrient levels and so on and in this way try to present a normal environment for the kidney.