In situ kidney preservation for transplantation with use of profound hypothermia (5 to 20° C.) with an intact circulation


Twenty-seven anesthetized dogs were surface cooled at 4 to 6° C. or 15 to 20° C. Circulation was provided by the mechanical ventricular assist in 23 dogs. After 24 or 48 hours of in situ preservation, the kidneys were transplanted into the necks or iliac fossas of anephric recipients. Renal function was preserved in the cadaver for up to 48 hours at 15 to 20° C. by maintaining a pulsatile circulation. Further cooling to 4 to 6° C. caused progressive deterioration in renal function. The nonperfused kidneys kept in situ at 4 to 6° C. did not produce any urine after transplantation. If the practical problems of total body cooling are solved, in situ preservation of multiple organs in the cadaver would increase the number of available organs for transplantation.

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The future of organ transplantation hinges on the maximal use of cadaver organs. Extracorporeal perfusion systems are capable of maintaining viability of single isolated organs for limited periods of time. Preservation of several organs in a single cadaver would increase the number of available organs and might simplify short-term storage.

Previous work from this laboratory has shown that circulation in dogs can be maintained effectively at very low temperatures (5° C.) with the mechanical ventricular assistance. The circulation in such a profound induced-hypothermia preparation has many characteristics of deep hibernation. With the same system dogs can be cooled to 5° C., rewarmed, and resuscitated with long-term survival. The effectiveness of this preparation for renal preservation was studied in dogs at 5 and 20° C.

MATERIAL AND METHOD

Twenty-seven beagle or mongrel dogs weighing 10 to 25 kilograms were anesthetized with sodium thiamylal (3 to 8 ml. of 2.5 percent solution) and succinylcholine (2.5 mg. per kilogram body weight) and cooled in an ice bath according to the method previously described. When effective cardiac action ceased (at 16 to 24° C.), a left thoracotomy was performed and the circulation was maintained with the mechanical ventricular assist (MVA) device placed on the surface of the heart. Ringer’s lactate solution was administered to all animals to maintain a central venous pressure above 2 cm. H2O and a hematocrit below 40 percent. Magnesium sulfate (2 ml. of a 50 percent solution) was added to every 500 c.c. of fluid administered after the temperature fell to below 20° C. Methylprednisolone, 5 mg. per kilogram of body weight, and chlorpromazine, 1 mg. per kilogram of body weight, were administered intravenously every 8 hours. Blood pressure, central venous pressure, esophageal temperature, and urine output were monitored continuously. Respiration were controlled with a volume ventilator delivering 12 ml. per kilogram of volume at a rate of 5 per minute and a gas mixture of 95 percent oxygen and 5 percent carbon dioxide.

The animals were studied in five groups: Group I—24 hour control preservation (no circulation) at 4 to 6° C. (four dogs); Group II—24 hour MVA preservation at 4 to 6° C. (11 dogs); Group III—48 hour MVA
preservation at 4 to 6°C. (four dogs); Group IV—24 hour MVA preservation at 15 to 20°C. (four dogs); Group V—48 hour MVA preservation at 15 to 20°C. (four dogs).

After 24 or 48 hours of hypothermia, a left nephrectomy was performed and the kidney was transplanted to the neck of anephric recipient dogs in 23 instances. A cutaneous ureterostomy was performed and the recipient was treated with intramuscular penicillin. Urine output and the development of uremia in the recipient were noted. All 23 recipients were killed on the third or fourth day after transplant day to avoid complications of rejection or infection. The contralateral right kidney of the donor dog was excised for histologic study in all instances.

Four kidneys stored at 15 to 20°C. (three for 24 hours and one for 48 hours) were transplanted into the right iliac fossa and the donor ureters were reimplanted into the recipients’ bladders. Bilateral nephrectomy then was performed. These four dogs received immunosuppressive therapy (azathioprine, 2 mg. per kilogram per day, and prednisolone, 0.4 mg. per kilogram per day) and long-term study of their renal function was done. The blood urea nitrogen (BUN), serum creatinine, and phenolsulfonphthalein (PSP) excretion were studied in all recipients.

RESULTS

Circulatory dynamics during profound hypothermia. As the animal cooled, the blood pressure and the heart rate dropped and, following cardiac resuscitation with the MVA, the mean blood pressure at 15 to 20°C. was 85 ± 20/45 ± 15 mm. Hg. This value dropped to 60 ± 18/31 ± 15 mm. Hg at 24 hours and to 40 ± 16/21 ± 8 mm. Hg at 48 hours.

When the temperature reached 5°C., the mean blood pressure was 41 ± 16/22 ± 10 mm. Hg and at 24 hours it dropped to 20 ± 15/10 ± 5 mm. Hg. After 48 hours at 5°C. on the MVA, there was a further drop in the mean blood pressure to 16 ± 9/8 ± 3 mm. Hg. Pulsatile flow was visible in all renal arteries of dogs supported by the MVA at the time of kidney harvesting.

Renal function. As the body temperature fell, the kidney lost its ability to concentrate urine and a variable initial diuresis occurred. Anuria generally developed below 25°C., although five dogs continued to produce small quantities of urine even at 5°C., provided that the mean blood pressure was maintained at the reasonably high level (over 50 to 60 mm. Hg) with the MVA, the mean blood pressure at 15 to 20°C variable (0 to 15 ml. per hour) and it decreased with time. Urine production continued for a longer period on the MVA at 20°C. (6 to 12 hours) than at 5°C. (2 to 5 hours).

Results of renal transplantation.

Group I—24 hour preservation at 5°C. with no circulatory support. The four control kidneys flushed with difficulty indicating increased vascular resistance. Return of color to the kidney after revascularization was slow and marked edema with engorgement developed over the first 5 minutes. No urine was produced by the four kidneys. BUN in the recipients rose to 155 mg. per 100 ml. (range, 145 to 175 mg. per 100 ml.) on the second postoperative day (Fig. 1). The serum creatinine rose to 6.3 mg. per 100 ml. (range, 4.9 to 8.0 mg. per 100 ml.). All four animals were clinically uremic. The PSP excretion was uniformly low and ranged from 0 to 5 percent in one hour. Histologic examination of the kidneys revealed extensive edema of the tissues, tubular damage, and vascular disruption and thrombosis.
Group II—24 hour preservation at 5°C with MVA. All eleven kidneys were transplanted successfully with immediate production of urine within 5 to 10 minutes of completion of the vascular anastomoses. The rate of urine production was small (2 to 15 ml per hour) and variable. BUN and creatinine rose progressively, but at a slower rate than in control dogs (Fig. 2). On the second postoperative day, BUN was significantly lower in nine MVA dogs than in control dogs. The mean BUN in Group II dogs was 68 mg per 100 ml with a range of 30 to 90 mg per 100 ml. Similarly the range of serum creatinine level on the second postoperative day was 3.1 to 4.8 mg per 100 ml. The mean PSP excretion was 38 percent (range, 3 to 61 percent) in one hour. Histology of Group II kidneys revealed variable degrees of edema with some damage to tubules and vascular endothelium. The degree of edema and cellular disruption was significantly less than that seen in Group I kidneys.

Technical difficulty was encountered during storage and transplantation of two kidneys (Fig. 2). One had no circulation for over 4 hours due to breakage of the MVA cup and the other had an unsatisfactory anastomosis due to a double renal artery. Function in these two kidneys paralleled that of nonperfused controls.

Group III—48 hour preservation at 5°C with MVA. All four kidneys had immediate urine production and renal function was significantly better than that of control (Fig. 3). The pattern was similar to that seen in recipients receiving the 24 hour perfused kidneys. BUN at 2 days was significantly higher than in the previous group with a mean of 106 mg per 100 ml, the range being 95 to 125 mg per 100 ml. The serum creatinine was 5.4 percent with a range of 4.2 to 5.8 percent. The mean PSP excretion was 30 percent (range, 18 to 47 percent) in one hour. Renal histology was similar to that in Group II, although the degree of edema and vascular damage was significantly more pronounced.

Groups IV and V—preservation at 15 to 20°C with the MVA. All four kidneys (one preserved for 24 hours and
three for 48 hours) had immediate urine output after transplantation into the neck of the recipients. They continued to function well throughout the 4 days of study. The BUN rose slightly and on the second day after transplant was 42 mg. per 100 ml. with a mean of 31 to 49 mg. per 100 ml. The serum creatinine at the time was 2.6 percent with a range of 1.8 to 3.1 mg. per 100 ml. The mean PSP excretion was 69 percent (range, 52 to 71 percent) in one hour. Histological examination revealed minimal amount of edema with scanty tubular and vascular damage. Most of the kidneys had virtually normal microscopic appearance.

Groups IV and V—long-term renal function after 24 or 48 hours preservation at 15 to 20°C. Four kidneys (three for 24 hours and one for 48 hour preservation) were implanted in the iliac fossas and the recipients were treated with azathioprine and prednisolone. Blood urea nitrogen and creatinine levels were elevated mildly for the first 10 days, but fell to within normal limits around the fourteenth postoperative day, at which time all animals were active and clinically well (Figs. 4 and 5). All four dogs died of generalized sepsis between the twenty-third and thirty-sixth days after transplant due to the immunosuppressive regimen.
DISCUSSION

Marchioro and associates\(^2\) first introduced the concept of restoring the circulation immediately after death to permit cadaver organ preservation. They employed standard cardiopulmonary bypass techniques and showed that livers and kidneys preserved by this method could be transplanted successfully. However, hypothermia to below 10\(^\circ\) C. was uniformly unsuccessful as an adjunct to their continuous perfusion technique.

Skinner, Newman, and Squire\(^4\) demonstrated that canine organs were preserved in vivo in a functioning state for at least 24 hours at normothermia after spontaneous heart action ceased if pulsatile circulation were restored and maintained by the MVA. Successful human kidney allotransplantation after 6 hours of cadaver preservation by the MVA at normal body temperature was reported.\(^3\) Addition of total body hypothermia to this pulsatile flow system might increase the viable period significantly and allow sufficient time for tissue typing, recipient identification, and preparation.

Our results show that maintaining a pulsatile circulation with the MVA during profound induced hypothermia preserves renal function to a varying degree. Good kidney function has been demonstrated after 48 hours of cadaver storage with moderate hypothermia at about 15 to 20\(^\circ\) C. Further cooling to 5\(^\circ\) C. caused progressive deterioration of cellular function which can be explained by the cold sensitivity of cation transport in kidneys below 10\(^\circ\) C.\(^3\)

Excellent immediate and long-term results have been reported after renal preservation for up to 72 hours on the Belzer machine.\(^1\) Comparable success has not been achieved yet with in vivo cadaver preservation. We cannot therefore recommend our method for human cadaver preservation at present. Total body cooling poses numerous practical problems. The value of core cooling as opposed to surface cooling is not settled. The significance of donor cryoglobulins and cryofibrinogens which may be precipitated during in vivo organ preservation is ill understood. The theoretical advantages, however, indicate that these lines of investigation may provide a solution to multiple organ preservation from a single donor.

In our institution the MVA is being used currently in the resuscitation of patients who do not respond to conventional measures. Kidneys are being preserved in situ for up to 6 hours at normal body temperature. Blood pressure, urine output, blood gases, pH, and electrolytes are monitored continuously. Any acidosis is corrected by intravenous sodium bicarbonate. The use of the MVA in this fashion to maintain a circulation after death permits tissue typing while blood flow to the kidney is maintained. It allows evaluation of renal function and biochemical adjustment in the donor to ensure optimal perfusion before kidney removal. Whether moderate body cooling to about 20\(^\circ\) C. with the MVA will prove to be an efficient method of long-term cadaver preservation remains to be proved.

REFERENCES