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Functional Analysis of Hamster Kidney Xenografts in the Rat: Possible Functional Incompatibility and Adaptation of Hamster Kidney Grafts in a Xenogenic Rat Environment

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XENOGRAFT function in a species-different environment will be a concern when the immunological barriers to xenotransplantation are overcome. In this study, long-surviving hamster kidney graft function in Lewis rat recipients was studied.

MATERIALS AND METHODS

Transplant Procedure and Immunosuppressive Agents

Orthotopic kidney transplantation was performed from golden Syrian hamsters (Charles River Lab, Wilmington, Mass) to inbred male Lewis rats (LEW, Harlan Sprague Dawley Inc, Indianapolis, Ind). The surgical procedure for kidney transplantation has been described elsewhere.[1] Briefly, vascular anastomosis was performed between the end graft aorta in continuity with the left renal artery and the recipient infrarenal aorta, and the graft left renal vein and the inferior vena cava. The donor bladder patch was sewn to the recipient bladder. Bilateral native nephrectomy was performed at the time of transplantation. Tacrolimus (Fujisawa pharmaceutical Co Ltd, Osaka, Japan) was given at a intramuscular dose of 1 mg/kg/d on days 0 to 30, followed by 0.5 mg/kg/every other day. Cyclophosphamide (CP) was given at an oral dose of 15 mg/kg/d for 5 days (day −3 to 1).

Animal Care and Sampling

Each recipient was maintained in a metabolic cage for at least 7 days prior to operation to allow them to adapt to the environment. After transplantation, the recipient was kept in the metabolic cage every other day for 30 days and weekly thereafter to measure water uptake and urine volume, and to collect urine samples. A blood sample (0.5 ml) was taken from the tail vein one day after grafting, weekly thereafter, and at the time of death. Renal function was evaluated by measuring creatinine, blood urea nitrogen (BUN), protein, Na, Cl, K, and osmolality in urine and serum samples. Osmolality was estimated by measuring the freezing point depression of a solution (urine or serum) using an automatic osmometer (Osmette A, Precision System Inc).

RESULTS

Median graft survival under Tacrolimus and CP treatment was 76 days (n = 12), and four animals survived for more than 100 days. These results were comparable to our previous observations.[1] After an initial loss during the early postoperative period, recipient animals maintained stable body weight for 8 to 10 weeks after transplantation. The daily urine excretion and water uptake increased rapidly after xenotransplantation and maintained four to sixfold more daily urinary excretion (53.5 ± 8 mL at 30 days after transplantation) for several weeks, compared to those of hamster and Lewis rats (both 9.3 ± 1.3 mL; Table 1). Urinary osmolality and creatinine and Na levels were significantly reduced in transplanted animals. However, total daily excretion of creatinine and Na was comparable

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Table 1. Osmolality and Creatinine and Na Levels in Urine and Serum Samples Obtained From Normal Rats, Hamsters, and Transplanted Rats

	Normal LEW (n = 12)	Normal Hamster (n = 4)	Xeno Tx Rats d 30–40 (n = 5)	Iso Tx Rats d 30–40 (n = 2)
Body weight (g)	230–260	130–140	210–240	240–280
Water uptake (mL/24 h)	15.5 ± 3.6	11.8 ± 1.3	60.0 ± 12.8	24.7 ± 6.2
Urine volume (mL/24 h)	9.3 ± 1.3	9.3 ± 1.3	53.5 ± 8	17.3 ± 1.9
Urine				
Creatinine (mg/dL)	137.3 ± 10.4	23.6 ± 7.1	15.4 ± 5.1	62.1 ± 6.8
Na (mEq/L)	169.4 ± 16.2	108.7 ± 27.7	52.5 ± 17.8	68.7 ± 14.3
Osmolality (mOsm/kg H_2O)	2233 ± 41	1661 ± 221	560 ± 34	1442 ± 35.7
Serum				
Creatinine (mg/dL)	0.77 ± 0.17	0.48 ± 0.07	0.97 ± 0.21	1.02 ± 0.08
Na (mEq/L)	151.7 ± 13.1	134.7 ± 15.3	145.3 ± 11.1	NT*
Osmolality (mOsm/kg H_2O)	311 ± 4	305 ± 5	325 ± 25	NT

*NT, not tested.

to that of normal rats. Serum levels of creatinine and Na and osmolality remained normal (Table 1).

Restriction of water intake reduced urine volume 50% to 60% and increased urinary osmolality 20% in normal rats and hamsters, compared to those with water intake *ad libitum*. However, water restriction did not affect relative polyuria (1.76 ± 0.57 to 1.39 ± 0.21 mL/h) or urinary osmolality (524.53 ± 54.07 to 506 ± 26.29 mOsm/kg H_2O) in xenotransplanted animals.

Arginine-vasopressin administration (1000 mU subcutaneous injection, every 12 hours for 5 days) reduced daily urine excretion by a maximum of 10% to 15% in rats and 20% to 60% in hamsters. The same treatment significantly reduced urine volume for 40% to 60% in transplanted animals. Urinary osmolality increased 10%, 50%, and 25% to 35% in rats, hamsters, and transplanted recipients, respectively.

Histopathological examination of kidney xenografts at day 30 revealed the existence of focal mild arterial inflammation and intimal fibrosis. Immunofluorescent examination showed faint rat IgM > IgG deposits in the glomerulus of these kidneys.

DISCUSSION

Persistent polyuria was seen after hamster to rat kidney xenotransplantation without significant changes in serum Na or osmolality. The most likely cause of this problem is a partial nephrogenic diabetes insipidus that may be related to xenotransplantation itself. An osmotic diuresis due to hyperglycemia has been ruled out. A functional mismatch between the hamster kidney and the rat's ADH may be the underlining process. This also may be complicated by the existence of mild histopathological evidence of rejection. The partial increase in urine osmolality after pharmacological dose of ADH suggests that the transplanted kidney is not totally refractory to ADH. Another possible cause includes a central abnormality in thirst mechanism leading to excessive fluid intake and resulting high-volume urine output and washout of the concentration gradient in the kidney. Additional studies should be undertaken to explore the precise cause of polyuria. Regardless of the ultimate mechanism, xenotransplantation in this model has led to an unsuspected functional derangement in renal physiology which may be related to differences in the normal renal physiology of each species.

REFERENCES

1. Miyazawa H, Murase N, Demetris AJ, et al: Transplantation 59:1183, 1995