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Histological Alterations Found in the Ureter During Organ Preservation and Early Phases of Renal Transplantation

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ABSTRACT

Introduction. There are no studies on the phenomena that occur on the ureter during organ preservation and immediately after transplantation.

Material and methods. We studied ureteral fragments obtained during organ harvesting in the cadaver ($n = 9$), after cold preservation period ($n = 18$), and immediately after kidney graft reperfusion ($n = 126$). In addition to the histological analysis, we evaluated the risk factors for the development of lesions and their relation to the evolution of the transplant.

Results. Alterations were detected in 120 of the 126 fragments studied after graft reperfusion. Global cellular infiltration was considered to be normal, mild, and moderate to severe in 34.9%, 41.3%, and 23.8%, respectively, consisting mainly of $CD8^+$ T lymphocytes. Urothelial exfoliation and cell vacuolization were detected in 42% and 52.4% of the cases, respectively. There was an inverse relationship between donor ventilation time and the intensity of the cellular infiltration. Seven and three of the nine fragments obtained during organ harvesting showed mild cellular infiltration of the chorion and urothelium, respectively. Cold storage promoted minor histological changes. After reperfusion, there was increased urothelial infiltration in 11 of the 18 cases. There was no relation between the lesions encountered and human leukocyte antigen compatibilities, renal rejections episodes, or the evolution of the graft itself.

Conclusions. Consequences of brain death mechanical ventilation were detected at the ureteral level, with abnormal lymphocytic infiltration in most cases. Cold storage did not produce any major histological changes. The lesions detected after graft reperfusion do not seem to involve immunological phenomena.

Despite extensive investigations on kidney preservation and transplantation, there are no studies on the phenomena that occur in the ureter during organ preservation and immediately after transplantation. In addition to the immunological aggression of allotransplantation, the surgical manipulation, the use of preservation solutions, and the cold ischemia period are potential additional insults, whose effects are not established. We studied the histological alterations in the ureter in the cadaveric donor, after organ preservation, and after graft reperfusion to evaluate risk factors and implications in the evolution of the transplant.

MATERIALS AND METHODS

We studied 126 ureteral fragments obtained 10 minutes after kidney graft reperfusion, taken from the segment of the ureter that is usually discarded at reimplantation. All but one graft was from a cadaveric donor. The mean age of the donors was 32.25 ± 15.30 years (7 to 72 years). The cause of death of cadaveric donors was head trauma in 99 cases, brain stroke in 17 cases, brain tumor in four cases, and other causes in five cases. The mechanical ventilation time was 69.94 ± 66.51 hours (8 to 288 hours). All grafts were perfused with University of Wisconsin solution and cold ischemia time was 20.54 ± 4.89 hours (1.30 to 39.00 hours). Recipient age was 39.57 ± 13.70 years (9 to 65 years), mean dialysis time was 32.19 ± 30.87 months (0 to 166 months) and human leukocyte

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antigen (HLA) compatibilities were 2.77 ± 0.98 (0 to 6). From the same pool of 126 transplant cases, we further studied nine fragments obtained during cadaveric organ harvesting and another 18 collected immediately after cold preservation. All fragments were fixed in formalin and stained with eosin-hematoxylin, CD3, CD4, CD8, and, when justified, Masson trichrome and Martinotti stain. We evaluated the cellular infiltration of the urothelium, chorion, and muscular layer, characterizing its grade and type and registering the presence of urothelial vacuolization and exfoliation. Taking into account the criteria for classification of transplant lesions in several organs[1–3] and the work of Paccione et al.[4] we developed criteria for classification of the ureteral lesions, including the presence of acute rejection. We analyzed the donor, recipient, and transplant risk factors for the lesions and for their evolution including the occurrence of ureteral complications and renal rejection episodes. The Student *t*, ANOVA, and χ^2 tests were used for statistical analysis; for all tests, a *P* value <.05 was considered significant.

RESULTS

All fragments allowed proper histological study. One hundred twenty of the 126 fragments studied after graft reperfusion showed some alteration from normal ureteral histology, namely epithelial exfoliation in 42%, cell vacuolization in 52.4%, and cellular infiltration of the chorion and urothelium in 74.6% and 77.8%, respectively. Cellular permeation of the urothelium was identified in 98 (87.8%) fragments, exclusively by T lymphocytes. Global cellular infiltration (muscle, chorion, and urothelium) was considered to be normal, mild, and moderate to severe in 34.9%, 41.3%, and 23.8%, respectively, consisting mainly of $CD8^+$ lymphocytes. There was an inverse relationship between donor ventilation time and the intensity of the cellular infiltration (Table 1).

Donor age and HLA compatibilities were higher among the cases of moderate to severe infiltration compared to normal (Table 1). No other significant relationship to risk factors was detected; cellular infiltration did not appear to relate to graft evolution (Table 2). There was no detectable relation between the lesions encountered and the evolution of the graft itself. Regarding urothelial exfoliation and vacuolization, the only relation detected was between younger donor age and vacuolization (28.91 ± 13.06 years vs 35.92 ± 16.79 years, $P = .011$).

Seven and three of the nine fragments obtained during organ harvesting showed mild cellular infiltration of the chorion and urothelium, respectively, mainly by $CD8^+$ T lymphocytes. Cold storage promoted minor histological changes, apart from lymphocytic migration to the epithelium. A longer cold ischemia time was related to the presence of urothelial infiltration before reperfusion (24.15 ± 2.90 hours vs 19.70 ± 3.96 hours, $P = .020$). Comparison with the fragments obtained after the period of cold ischemia showed that lymphocytic urothelial infiltration was more pronounced after reperfusion, with an increase in 11 of the 18 cases. No significant risk factors were detected for this finding. Recipient age was an average of 10 years less in the cases of increased infiltration, but the difference did not reach statistical significance, probably due to the low numbers (35.27 ± 11.55 years vs 45.14 ± 13.28 years, $P = .115$).

DISCUSSION

The urothelium is thought to be devoid of lymphocytes,[5] despite some reports in the contrary.[6] In this study, the detection of lymphocytes in the urothelial fragments obtained before reperfusion may be related to the fact that the donors were dead, in concordance with the work of Hoffman et al[7] and of Koo et al,[8] who observed higher cellular infiltration in renal biopsies from cadaveric donors compared with living donors. The inverse relationship between mechanical ventilation length and intensity of cellular infiltration may be related to the fact that mechanically ventilated trauma patients, after an initial rise in the immune

Table 1. Risk Factors From the Donor and Recipient for Lymphocytic Infiltration

	Lymphocytic infiltration			
	Normal (*n* = 44)	Mild (*n* = 52)	Moderate/severe (*n* = 30)	*P*
Donor age (year)	28.45 ± 13.50	33.00 ± 15.71	36.0 ± 16.21	NS*
Donor ventilation time (hour)	82.32 ± 70.07	74.84 ± 73.89	42.72 ± 33.62	.035
Cold ischemia time (hour)	20.42 ± 4.87	20.61 ± 3.90	20.59 ± 6.42	NS
Recipient age (year)	39.98 ± 15.55	39.52 ± 13.02	39.07 ± 12.33	NS
Dyalisis time (month)	36.35 ± 35.62	30.02 ± 29.06	30.00 ± 26.95	NS
HLA compatibilities	2.50 ± 0.98	2.85 ± 0.96	3.03 ± 0.96	NS†
Donor cause of death (*n* = 125)				
Brain trauma	38	38	23	NS
Brain stroke and other	6	14	6	
Recipient-associated pathology				
No	20	31	18	NS
Yes	24	21	12	
Transplant surgery time				
<3 hours	38	46	27	NS
>3 hours	6	6	3	

*$P = .075$; difference between "normal" and "moderate/severe," $P = .023$.
†$P = .054$; difference between "normal" and "moderate/severe," $P = .023$.

Table 2. Relation Between Lymphocytic Infiltration and Transplant Evolution

	Lymphocytic infiltration			
	Normal (n = 44)	Mild (n = 52)	Moderate/ severe (n = 30)	P
Delayed graft function (n = 123)				
No	35	45	26	NS
Yes	7	6	4	
Renal acute rejection* (n = 102)				
No	28	32	21	NS
Yes	5	11	5	
Chronic graft dysfunction* (n = 99)				
No	30	35	23	NS
Yes	4	4	3	
Creatinine 1st year* (n = 100)				
≤1.2 mg/dL	16	15	12	NS
>1.2 mg/dL	17	26	14	

*Cases with more than 1 year of follow-up only.

response, show a decline, affecting mainly cellular immunity.[9]

The effects of cold preservation on the ureter have not been properly studied. Riehman et al studied the effects on the ureter of cold preservation for variable times in the guinea pig, but they only addressed biomechanics and contractility properties.[10] In our work, cold storage promoted minor alterations in the nine fragments obtained from cadavers, a finding similar to the experience of Koo et al in renal biopsies from five cases of cadaveric donor transplantation.[8] The tendency to a lower recipient age in the cases where there was an increased lymphocytic infiltration after reperfusion may denote a quicker and more intense graft revascularization in these cases, favoring cellular infiltration from the recipient. We did not study the origin of the infiltrating cells after reperfusion, but there is some evidence that they come from the recipient.[8] The lack of relation to the number of HLA compatibilities suggests that this cellular influx does not seem to involve an immunological phenomena. This is in accordance with the study of Dragun et al who, in the rat, performed renal biopsies after syngeneic and allogeneic renal transplantation with and without immunosuppression and found no significant differences,[11] and with the findings Hoffman et al[7] and Koo et al[8] in postreperfusion renal biopsies in the clinical setting. Cellular vacuolization is known to occur in renal tubular cells after reperfusion[7] and Katz et al[12] considered urothelial vacuolization to be a sign of rejection of the ureter. However, these lesions occur in the absence of rejection[13] and, in this study, were detected before reperfusion.

In conclusions, consequences of brain death or mechanical ventilation were detected at the ureteral level with abnormal lymphocytic infiltration in most cases. On the other hand, cold storage produced minor histological changes. The lesions detected after graft reperfusion do not seem to involve any immunological phenomena.

REFERENCES

1. Racusen LC, Solez K, Colvin RB, et al: The Banff 97 working classification of renal allograft pathology. Kidney Int 55:713, 1999
2. Kuusanmäki P, Halttunen J, Paavonen T, et al: Acute rejection of porcine small bowel allograft: an extended histological scoring system. Transplantation 58:757, 1994
3. Demetris AJ, Batts KP, Dhillon AP, et al: Banff schema for grading liver allograft rejection: an international consensus document. Hepatology 25:658, 1997
4. Paccione F, Enein AA, Shikata T, et al: Changes in the transplanted ureter. Brit J Exp Path 46:519, 1965
5. Jost SP, Gosling JA, Dixon JS: The morphology of normal human bladder urothelium. J Anat 167:103, 1989
6. El-Demitry MIM, Hargreave TB, Busuttil A, et al: Immunohistochemical identification of lymphocyte subsets and macrophages in normal human urothelium using monoclonal antibodies. Brit J Urol 58:436, 1986
7. Hoffmann SC, Kampen RL, Amur S, et al: Molecular and immunohistochemical characterization of the onset and resolution of human renal allograft ischemia-reperfusion injury. Transplantation 74:916, 2002
8. Koo DDH, Welsh KI, Roake JA, et al: Ischemia/reperfusion injury in human kidney transplantation: an immunohistochemical analysis of changes after reperfusion. Am J Pathol 153:557, 1998
9. Menges T, Engel J, Welters I, et al: Change in blood lymphocyte populations after multiple trauma: association with posttraumatic complications. Crit Care Med 27:733, 1999
10. Riehmann M, Rhodes PR, Ametani MS, et al: Preservation of bladder tissue in different solutions for varying times. Urology 50:142, 1997
11. Dragun D, Hoff U, Park JK, et al: Ischemia-reperfusion injury in renal transplantation is independent of the immunological background. Kidney Int 58:2166, 2000
12. Katz JP, Hakki A, Katz SM, et al: Rejection of the ureter: a new component of renal allograft rejection. Transplant Proc 19: 2200, 1987
13. Blasco-Casares FJ, Saladié-Roig JM, Encabo-Mayoral B, et al: Experimental assessment of ureteral free autograft. Urol Res 25:131, 1997