Transplant Int (1989) 2: 117-120

TRANSPLANT
International

Influence of different preservation solutions and intentional hemodilution of recipient on viability of preserved and transplanted rat kidneys

J. Jacobsson[1], J. Wahlberg[2], and G. Tufveson[2]

[1] Department of Surgery and [2] Department of Urology Transplantation Unit, University Hospital, S-75185 Uppsala, Sweden

Abstract. A total of 81 rat kidney grafts, flushed out and cold stored in either Sacks' or University of Wisconsin (UW) solution, were transplanted into hemodiluted (Hct = 30% ± 4%) or untreated (Hct = 43% ± 3%) recipients. The cold ischemia times (CIT) used were 24 and 36 h. One week after transplantation, the surviving recipients ($n = 67$) were contralaterally nephrectomized. The experiment was terminated after a total period of 4 weeks, and the percentage of surviving animals was determined for each treatment. Data was pooled and the results show that grafts cold stored in UW solution were viable to a significantly greater extent and after longer CIT than grafts cold stored in Sacks' solution (47% vs 23%; $P < 0.05$). Recipient hemodilution did not improve graft viability (39% vs 32%; NS). Kidneys cold stored for 24 h were viable to a greater extent than kidneys with a CIT of 36 h (50% vs 15%; $P < 0.01$).

Key words: Preservation solution - Hemodilution in kidney preservation.

The recently developed University of Wisconsin (UW) solution [8] has been shown to be useful in the preservation of several organs. UW solution has been used successfully to preserve canine renal [6] and pancreatic grafts [8, 9] for as long as 72 h and canine livers for up to 30 h [4]. UW solution contains high-molecular-weight anions and uncharged impermeants that reduce cold ischemia-induced tissue edema.

Offprint requests to: G. Tufveson

Recipient hematocrit status is an important factor in reperfusion injury, as hemodilution appears to reduce reperfusion damage to kidneys subjected to warm ischemia [2]. Recipient hematocrit is particularly important after the introduction of recombinant human erythropoietin (EPO) in the treatment of anemia in dialysis patients [11].

In an earlier publication, we reported results from acute transplantation experiments in which a modified UW (mUW)[1] solution was found to be more effective than Sacks' solution for cold storage of rat kidneys. We also found hemodilution of the recipient to result in further improvement [3]. These results were obtained by measuring the amount of erythrocyte trapping in the graft after 20 min of reperfusion.

The aim of the present study was to compare the results obtained from the acute transplantation model with the outcome of a graft viability model. Renal transplants were performed after 24 and 36 h of cold storage of the grafts in Sacks' or UW solution. The kidneys were then transplanted into hemodiluted or untreated recipients. Graft viability was assessed by animal survival after delayed contralateral nephrectomy.

Materials and methods

Inbred male Sprague-Dawley rats, weighing 200-260 g, were used for the experiments. The rats had free access to water and standard pellets (R3, ALAB, Sollentuna, Sweden). Anesthesia was induced by an intraperitoneal injection of chloralhydrate in a dose of 360 mg/kg body weight.

[1] mUW solution is UW solution from which the starch and additives have been removed. Reperfusion injury and animal survival after cold storage of rat kidneys in mUW is equal to that after cold storage in UW (Wahlberg et al., Transplantation, in press)

Table 1. Animal survival and hematocrit of the different treatment groups. S, Sacks' solution; UW, University of Wisconsin solution; CIT, cold ischemia time

Group	Preservation solution	CIT (h)	Number of rats		Hematocrit
			Contralaterally nephrectomized	Surviving	
1A	S	24	10	3	44±1
1B	S	24	10	4	29±3
2A	UW	24	9	6	44±3
2B	UW	24	11	7	29±3
3A	S	36	6	0	41±3
3B	S	36	5	0	28±2
4A	UW	36	6	1	42±2
4B	UW	36	10	3	31±4

Kidney harvesting procedure

The donor was tracheostomized. One hour before harvesting, the donor was pretreated with an IV injection of phenoxybenzamine in a dose of 3 mg/kg body weight. Ten minutes before harvesting, heparin was given IV in a dose of 300 IU. The left kidney was mobilized and a catheter was introduced into the aorta for in situ flush-out with either UW solution [8] or Sacks' solution [7]. The flush-out was continued for 10 min at a hydrostatic pressure of 70-80 cm water. The kidney was then excised and stored in the preservation solution at +4.0° ± 0.1°C until transplanted.

Transplantation procedure

The left kidney of the recipient was mobilized and removed after clamping of the renal artery and vein using microsurgical vessel clamps. These vessels were then prepared using a "cuff technique", described elsewhere [5]. The graft was placed in a Lucite cup filled with crushed ice to avoid warming during anastomosis. Transplantation was then performed by slipping the graft vessels over the prepared cuffs and tying the vessels in position. This anastomotic technique allowed the transplantation to be performed within 10 min. The ureter of the recipient was cut close to the kidney, where the diameter of the ureter is the largest. With the aid of forceps, the ureter was turned inside out so that a "collar" was formed. The wall of the donor ureter was anastomosed by two sutures to the edge of the collar. The collar was then pulled back to surround the donor ureter. Finally, the abdomen was closed.

Isovolemic hemodilution

Isovolemic hemodilution was performed 1 or 2 days prior to transplantation. It was accomplished by a simultaneous withdrawal of blood from the carotid artery and injection of a 5% human serum albumin solution (Kabi Vitrum, Stockholm, Sweden) into the jugular vein. The hematocrit values, determined at the time of transplantation, were 30%±4% for the hemodiluted recipients and 43%±3% for the untreated recipients. Hematocrit values returned to normal 1 week later (assayed in six animals).

Assessment of graft viability

One week after transplantation the rats were contralaterally nephrectomized. Rats that died before nephrectomy were classified as technical failures (17%). This mortality was evenly distributed among all experimental groups. The experiment was terminated 3 weeks after the delayed nephrectomy, and the percentage of surviving animals in each group was determined. Twenty-five percent of the transplanted rats that survived until the end of the experiment had hydronephrosis. This did not affect their serum urea (19.3±18 vs 18.1±4.9 mmol/1), and thus they were all included in the study.

Experimental groups

The rats were divided into four groups, depending on the solution used and the cold ischemia time (CIT). Each of these groups was subdivided into treated and untreated recipients. The numbers given within parentheses refer to the number of live, transplanted rats that underwent right kidney nephrectomy.

Group 1A ($n = 10$). Kidney grafts were flushed out and cold stored in Sacks' solution for 24 h and then transplanted into untreated recipients.
Group 1B ($n = 10$). The same as for group 1A but transplanted into hemodiluted animals
Group 2A ($n = 9$). Kidney grafts were flushed out and cold stored in UW solution for 24 h and then transplanted into untreated recipients.
Group 2B ($n = 11$). The same as for group 2A but transplanted into hemodiluted animals
Group 3A ($n = 6$). Kidney grafts were flushed out and cold stored in Sacks' solution for 36 h and then transplanted into untreated recipients.
Group 3B ($n = 5$). The same as for group 3A but transplanted into hemodiluted animals
Group 4A ($n = 6$). Kidney grafts were flushed out and cold stored in UW solution for 36 h and then transplanted into untreated recipients.
Group 4B ($n = 10$). The same as for group 4A but transplanted into hemodiluted animals

Statistics

Chi-square tests were used for comparison of group outcome (Table 2). To allow for meaningful analysis of this material, the animals were pooled for the three variables discussed: type of preservation solution, recipient hemodilution, and CIT.

Results

The results are presented in Table 1. UW solution was found to be better than Sacks' solution, regardless of recipient status. After a CIT of 24 h, preservation in Sacks' solution combined with recipient hemodilution resulted in a 40% survival rate (4/10 rats in group 1B); preservation in Sacks' solution followed by transplantation into untreated recipients resulted in a 30% survival rate (3/10 rats in group 1A). When UW solution and a CIT of 24 h were used, the survival rate was 64% in the hemodiluted group of recipients (7/11 rats in group 2B) and 67% for nonhemodiluted recipients (6/9 rats in group 2A).

Table 2. Chi-square calculations for preservation solution, recipient state, and cold ischemia time (CIT)

Perfusate	Outcome		Recipient state	Outcome		CIT	Outcome	
	Alive	Dead		Alive	Dead		Alive	Dead
Sacks' solution	7	24	Untreated	10	21	24 h	20	20
UW solution	17	19	Hemodiluted	14	22	36 h	4	23
	$P < 0.05$			$P =$ NS			$P < 0.01$	

No animals survived a CIT of 36 h when Sacks' solution was used for preservation and grafts were transplanted into hemodiluted or nonhemodiluted recipients (groups 3B and 3A, respectively). When UW solution was used and the grafts were cold stored for 36 h, 30% of the hemodiluted rats (3/10 in group 4B) and 17% of the nonhemodiluted ones (1/6 in group 4A) survived. This single surviving rat was sick and had lost weight, whereas all other surviving animals gained weight and appeared to be healthy. The only difference that reached statistical significance was that between the entire group of Sacks-preserved graft recipients and the entire group of UW-preserved graft recipients (Table 2; $P < 0.05$).

Shorter CIT (24 h) naturally showed better animal survival than longer CIT (36 h; $P < 0.01$). Recipient hemodilution, as such, seemed to have no beneficial effect on graft viability as assessed by this method, but the difference in hematocrit between the hemodiluted and nonhemodiluted recipients was only 13%.

Discussion

The method of assessing graft viability with 1-week delayed contralateral nephrectomy after transplantation allows the graft to recover from acute tubular necrosis. One may, therefore, conclude that most of the animal deaths were probably due to severe organ injury. Resistance to cold ischemia injury was better in this study than that previously observed after renal transplantation followed by acute contralateral nephrectomy under different, but seemingly satisfactory, preservation conditions in rats [1]. This suggests that a long CIT can be used with our model. Furthermore, the experimental design - delayed contralateral nephrectomy - resembles clinical renal transplantation, where temporary renal support can be accomplished with dialysis. The present study also confirms previous reports [4, 6, 8, 9] on the apparent advantages of using UW solution for organ preservation, even though the CIT used in those studies was considerably longer. The difference might be explained by a tolerance to cold ischemia that is lower in rats than in larger species. A comparison with the more commonly used Euro Collins solution was not possible since this solution failed to reproducibly perfuse our rat kidneys. Our results are also in agreement with our previous observation that mUW solution is better than Sacks' solution in preventing trapping of erythrocytes in the renal vasculature 20 min after reperfusion of rat kidney grafts injured by cold ischemia [3].

Recipient hemodilution, in combination with either Sacks' or mUW solution, decreased the trapping for all CIT tested in the acute model [3]. The best results were obtained when mUW solution was used for preservation and combined with grafting into hemodiluted recipients. However, we cannot confirm a beneficial effect of recipient hemodilution in the present study. One reason for the lack of positive effect of hemodilution may be the relatively high hematocrit ($30\% \pm 4\%$) achieved. This is higher than in our earlier series of trapping experiments, where the hematocrit was lowered to $25\% \pm 3\%$. Something else that may have contributed to the lack of effect of recipient hemodilution could be the fact that the hematocrit values returned to normal 1 week later.

Alternatively, the positive effect of recipient hemodilution is limited to the reperfusion phase; the kidneys recover from the injury caused by high hematocrit within the period before contralateral nephrectomy. Suggestive of this is the finding from a preliminary investigation of clinical renal transplant patient material, in which EPO-treated patients with a significantly higher hematocrit than untreated patients had immediate onset of graft function in only two out of nine cases, this in contrast to 70% immediate onset of function in the total patient material. However, at 3 weeks after transplantation, no obvious difference could be noted in the creatinine levels or graft survival between EPO-treated and untreated recipients [10]. The lack of a difference in the human material at 3 weeks is in accordance with the results of the present study.

Whereas the role of UW solution seems to be established and CIT still sets the limits for organ preservation, the exact role of recipient hemodilution needs further attention.

Acknowledgement. We wish to acknowledge the technical and skillful help of Ester Roos-Engstrand and Eva Sandström. We also wish to acknowledge the financial support we received from the Tore Nilsson Fund, the Fortia Fund, the Maud and Birger Gustavsson Fund, Njursjukas Förening CUWX, and the Swedish Medical Research Council.

References

1. Harvig B, Norlén BJ (1980) A technique for studies *in vivo* and *in vitro* on preserved and transplanted rat kidney. Urol Res 8: 107-112
2. Hellberg O, Andréen T, Bayati A, Källskog Ö, Wolgast M (1985) Pathophysiological significance of rheological factors in the development of acute renal failure. Acta Physiol Scand 124 [Suppl 542]: 382
3. Jacobsson J, Tufveson G, Odlind B, Wahlberg J (1989) Type of preservation solution and hemodilution of recipient affects postischemic erythrocyte trapping in kidney grafts: an experimental study in the rat. Transplantation, in press
4. Jamieson NV, Sundberg R, Lindell S, Laravuso R, Kalayoglu M, Southard JH, Belzer FO (1988) Successful 24- to 30-h preservation of the canine liver: a preliminary report. Transplant Proc 20 [Suppl 1]: 945-947
5. Olausson M, Mjörnstedt L, Lindholm L, Brynger H (1984) Non-suture organ grafting to the neck vessels in rats. Acta Chir Scand 150: 463-467
6. Ploeg RJ, Goosens D, McAnulty JF, Southard JH, Belzer FO (1988) Successful 72-hour cold storage of dog kidneys with UW solution. Transplantation 46: 191-196
7. Sacks SA, Petritsch PH, Escott ML (1973) Canine kidney preservation using a new perfusate. Lancet I: 1024-1028
8. Wahlberg J, Southard JH, Belzer FO (1986) Development of a cold storage solution for pancreas preservation. Cryobiology 23: 477-482
9. Wahlberg J, Love R, Landegaard L, Southard JH, Belzer FO (1987) 72-hour preservation of the canine pancreas. Transplantation 43: 5-8
10. Wahlberg J, Jacobsson J, Odlind B, Tufveson G, Wikström B (1988) Haemodilution in renal transplantation in patients on erythropoetin. Lancet II: 1418
11. Winearls CG, Pippard MJ, Downing MR, Oliver DO, Reid C, Cotes PM (1986) Effect of human erythropoietin derived from recombinant DNA on the anemia of patients maintained by chronic hemodialysis. Lancet II: 1175-1177