Urol Res (1988) 16:53–55

Urological Research

Failure to Prolong Rat Renal Allograft Survival Time by Photochemical Donor Kidney Pretreatment During Hypothermic Pulsatile Kidney Preservation

H. Oesterwitz[1], J. Kaden[1], W. Schneider[2] and W. Fritsch[1]

[1] Division of Experimental Organ Transplantation, Department of Urology and
[2] Institute of Pathology, Humboldt University (Charité), Berlin, German Democratic Republic

Accepted: January 15, 1987

Summary. Systemic photochemical pretreatment of a rat kidney donor with the photosensitizer 8-methoxypsoralen (8-MOP; 0.06 mg/kg intravenously, 10 min before graft removal) plus ex vivo longwave ultraviolet (UVA) irradiation of the kidney graft during simple hypothermic storage significantly prolonged survival time in allogeneic recipients. In contrast to these results, the present use of UVA irradiation during hypothermic pulsatile kidney perfusion using Euro-Collins® solution containing 8-MOP (0.06 mg/ml) did not prolong graft survival compared with untreated controls. Systemic application of 8-MOP to the kidney donor may be necessary for effective action of the combined photochemical treatment as a method of immunoalteration. The extended UVA irradiation time and the local use of 8-MOP in the preservation fluid had no effect on graft survival possibly because of inadequate tissue distribution of 8-MOP during both hypothermia and perfusion.

Key words: Renal transplantation – Rat – Ultraviolet irradiation – PUVA – Pulsatile perfusion

Introduction

A recent paper [7] showed that pretreatment of the rat kidney donor with 8-MOP followed by UVA irradiation of the graft (PUVA therapy) significantly prolonged survival in allogeneic recipients without further immunosuppression. Similar results were obtained in the rat heart allograft model [9]. The longterm survival of PUVA-treated rat renal allografts is mediated by both a strong reduction of graft immunogenicity – which probably represents the loss or depletion of highly immunogenic dendritic cells (DCs) – and the development of graft protecting humoral and cellular effectors [10].

The aim of the present study was to increase the effectiveness of PUVA therapy by extended UVA irradiation time and local application of 8-MOP to the kidney graft during hypothermic pulsatile perfusion.

Material and Methods

Animals

Inbred male Wistar rats (320–460 g) were used for pilot studies to choose the preservation fluid for pulsatile perfusion (Laboratory Animal Service, Schönwalde, GDR). In the allotransplant experiments inbred male Sprague-Dawley (SD) rats and BD IX rats (330–440 g) were used (Academy of Science of the GDR, Central Institute for Cancer Research, Berlin). SD rats (RT-1^u) served as kidney donors; BD IX rats (RT-I^d) acted as recipients. These strains differed at the major histocompatibility complex (MHC).

Kidney Transplantation

The microsurgical technique has been published in this journal [6]. Left orthotopic grafts with end-to-end anastomoses of the renal vessels and ureter were made. Right recipient nephrectomy was performed at the time of transplantation. Graft function was followed by serial blood urea nitrogen (BUN) estimations (day 2, 7, 10, 14, thereafter monthly). Autopsies were performed on most rats which were examined as reported previously [5].

Pilot Studies

In these investigations 2 different perfusates for pulsatile perfusion were tested to obtain an optimal protection from ischemic kidney damage in the allotransplant studies. In each case 5 syngeneic kidney transplantations were performed after pulsatile perfusion for 240 min using Euro-Collins® solution (group A) and an electrolyte solution containing 5% albumin (group B; Kabi Vitrum, Stockholm, Sweden) respectively. In group A all animals survived for more than 100 days with a slight increase of the BUN level (16 ± 2 mmol/l) at day 2 whereas all rats in group B died 10–32 days after transplantation with high BUN levels (>100 mmol/l) due to renal failure. Therefore, in the following experiments Euro-Collins® solution was used for kidney preservation. Five additional syngeneic transplants (group

Table 1. Experimental group design and results

Experimental groups/treatment	n	BUN at day 2 ($\bar{x}$, mmol/l)	Graft survival (days)
(1) untreated control	7	16	8, 8, 8, 9, 9, 9, 11
(2) PUVA	7	15	8, 8, 8, 9, 10, 12, 14

C) were performed after perfusion with Euro-Collins® solution containing 0.06 mg/ml of 8-MOP (Oxsoralen®, GEROT Pharmazeutika, Vienna, Austria) to detect any deleterious effect of 8-MOP on kidney function during perfusion. The results were the same as in group A.

Continuous Pulsatile Kidney Perfusion

Immediately after removal and initial flushing with child Euro-Collins® solution the kidney was placed in a special preservation machine [1]. The kidney was pulse perfused (frequency 80 ± 5 · min^{-1}) with Euro-Collins® solution for 200 ± 13 min at 8 ∠ 2 °C. The mean perfusion pressure was 50 ± 5 mmHg, the perfusion flow ranges drom 0.9–1.6 (mean 1.2) ml · min^{-1}.

UVA Irradiation

During hypothermic preservation the kidney grafts in group 2 were irradiated with a 40 W mercury arc medium pressure lamp (UVS 40-40-2, NARVA, Berlin, GDR) at a distance of 12.3 cm for 200 ± 13 min and thereafter transplanted. The UVA intensity was measured as 14 ± 0.91 J · cm^{-2}.

Experimental Groups

The experimental design is shown in Table 1. Seven untreated allografts (group 1) were transplanted after perfusion with pure Euro-Collins® solution. In group 2 seven allografts were transplanted after perfusion with Euro-Collins® solution containing 1.4 mg of 8-MOP (0.06 mg/ml). Recipients with elevated BUN levels (>20 mmol/l) on the 2nd day after operation as a result of technical complications (ureteral fistula, vascular thrombosis) were excluded from the study.

Results

The results in the experimental groups are summarized in the Table. In the untreated control group 1 the kidneys were rejected rapidly. The PUVA treatment during hypothermic pulsatile perfusion (group 2) did not improve graft survival time and all transplants were rejected in the 2nd post-transplant week.

Discussion

PUVA treatment of murine skin and rat kidney and heart allografts, respectively, resulted in reduced immunogenicity of the grafts and prolonged survival after transplantation on allogeneic recipients [2, 8, 9]. The efficiacy of this therapy was dependent on the time and dose of UVA irradiation [7]. A specific inhibition of MHC class II antigen expression by photochemical treatment was demonstrated [3]. The influence of photoirradiation on MHC class II antigens of intragraft antigen-presenting cells, e.g. interstitial DCs, may be an important reason for the prolongation of allograft survival by PUVA pretreatment [11]. In the same way the local allograft pretreatment of murine skin in a culture medium containing cyclosporin at 50 μg/ml [4] and of canine kidneys by flushing with Ringer's solution containing cyclosporin at 50 mg/ml [12], respectively, prolonged the transplant survival time. Reduction of graft immunogenicity has widespread clinical application and we attempted to increase the efficiacy of our photochemical donor and graft pretreatment protocol by a prolongation of the UVA irradiation time and by direct application of the photosensitizer in the perfusion fluid. This PUVA treatment protocol had a nullifying effect on rat kidney graft survival. The pulsatile perfusion per se using Euro-Collins® solution as well as the addition of 8-MOP to the preservation fluid was not deleterious to graft function. In conclusion, a systemic application of the photosensitizer to the kidney donor seems to be necessary for the effective subsequent action of the UVA irradiation. Therefore, further investigation should be directed to the combined photochemical treatment of both donor and the allograft.

References

1. Fritsch W, Kreuschner H, Kühn B, Oesterwitz H, Wischke UW (1985) Anordnung zur hypothermen Rattennierenperfusion. VI Symposium für Experimentelle Urologie, Rostock
2. Gruner S, Meffert H, Karasek E, Sönnichsen N (1984) Prolongation of skin graft survival in mice by in vitro PUVA treatment and failure of induction of specific immunological memory by PUVA-treated grafts. Arch Dermatol Res 276:82–85
3. Gruner S, Volk HD, Noack F, Meffert H, Baehr R von (1986) Inhibition of HLA-DR antigen expression and of the allogeneic mixed luekocyte reaction by photochemical treatment. Tissue Antigens 27:147–154
4. Gruner S, Oesterwitz H, Baehr R von (1986) Verlängerung der Überlebenszeit muriner Hauttransplantate durch eine Spendervorbehandlung mit Cyclosporin A. Z Klin Med (submitted for publication)
5. Kaden J, Oesterwitz H, Groth J, Schade R, Schneider W, Schröder K (1984) Humorale Immunantwort, Konzentration

ausgewählter Serumproteine und Transplantathistologie nach allogener Nierentransplantation bei Ratten. Z Urol Nephrol 77:457–463

6. Oesterwitz H, Althaus P (1982) Orthotopic kidney transplantation in the rat with non-splinted end-to-end ureteric anastomosis: details of a technique. Urol Res 10:149–152
7. Oesterwitz H, Scholz D, Kaden J, Mebel M (1985) Prolongation of rat renal allograft survuval time by donor pretreatment with 8-methoxypsoralen and longwave ultraviolet irradiation of the graft (PUVA therapy). Urol Res 13:95–98
8. Oesterwitz H, Kaden J, Scholz D, Mebel M (1986) Donor pretreatment with 8-methoxypsoralen followed by ultraviolet irradiation of the graft: a new approach to enhance rat renal allograft survival. Transplant Proc 18:1297–1298
9. Oesterwitz H, Schneider W (1986) Prolonged rat heart allograft survival by photochemical donor pretreatment. J Surg Res (submitted for publication)
10. Oesterwitz H, Kaden J, Schneider W (1987) Reduced immunogenicity of rat renal allografts after photochemical donor pretreatment and passive transfer of graft protection. Urol Res 15:207–210
11. Oesterwitz H, Kaden J, Schneider W, Schirrow R, Scholz D (1986) Synergistic effect of photochemical donor pretreatment and cyclosporin A therapy of the recipient on rat renal allograft survival. Nephrol Dial Transplant (submitted for publication)
12. Toledo-Pereyra LH, MacKenzie GH, Gordon DA, Toben HR (1982) Prolongation of kidney transplant survival by cyclosporin A graft pretreatment: initial experimental trial. Transplantation 33:329–330

Dr. H. Oesterwitz
Abteilung für experimentelle Organtransplantation
Humboldt-Universität (Charité)
Leninallee 49
DDR-1917 Berlin
German Democratic Republic