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Effects of PUVA therapy on kidney allografts: results of a randomized prospective double-blind study

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Abstract After successful experimental organ transplant studies on the efficacy of PUVA therapy combining donor pretreatment with the photosensitizer 8-methoxypsoralen (P) and the ex vivo irradiation of organs with long-wave ultraviolet light (UVA) prior to transplantation, we started in 1989 the first randomized, prospective, double-blind study to clarify the efficacy of PUVA therapy in human kidney transplantation. This study included 50 kidney donors, 25 of whom were PUVA-treated. A total of 75 kidneys were transplanted in Berlin, Halle and Rostock. The complete data of these 75 recipients were available for the final evaluation. The PUVA group ($n = 36$) and the non-PUVA group ($n = 39$) were not statistically significantly different as to donor and recipient data. Regarding the results, no differences were seen in initial hospitalization time, early graft function, rejection rate, number and time of rejection episodes. After a follow-up of 24 months, both graft survival (PUVA vs. non-PUVA: 75% vs. 71.8%) and patient survival (97.2% vs. 97.4%, respectively) were comparably high. PUVA therapy did not influence the development of vascular rejection. Interestingly, the rate of late graft loss after the 6th posttransplant month was lower, but not statistically significantly so, in the PUVA than in the non-PUVA-group (2 vs. 6 graft losses). Thus, PUVA-pretreated kidneys may be associated with a reduced development of chronic rejection.

Key words Kidney transplantation
PUVA · 8-Methoxypsoralen · UVA
Graft survival · Patient survival

Introduction

Stimulated by the results of Gruner et al. [4] and Lau et al. [12] demonstrating a beneficial effect of PUVA or UVB in murine skin and rat pancreatic islet allograft models, we tried to apply this method to vascularized organ transplant systems. In 1985, we reported on a significant prolongation of rat renal allograft survival time after PUVA therapy [13]. This kind of therapy included both the pretreatment of the donor with the photosensitizer

psoralen (P) and the subsequent long-wave ultraviolet radiation (UVA) of the removed kidneys during hypothermic preservation. After transplantation into bilaterally nephrectomized rats without any other immunosuppression, an indefinite graft survival was observed in 40% with a strain combination of strong MHC differences and in 90% with a semioallogeneic system using F1 hybrids as donors [1, 2].

The transplantation of PUVA-treated rat kidney allografts into temporary cyclosporine A (CsA)-immunosuppressed, different MHC recipients led to permanent survival of the graft in 70%, indicating a synergistic effect between PUVA and CsA [15]. In subsequent experiments we were able to prolong the survival time of rat heart allografts also by PUVA [18]. In immunohistological studies using monoclonal antibodies to rat MHC class I and II antigens (Serotec, UK), we could show a significant reduction of class II antigen expression in kidney as well as heart cryostat sections after PUVA treatment [2, 7]. After retransplantation of long-term surviving, PUVA-pretreated rat kidneys into naive rats, all of the 7 secon recipients survived for more than 100 days, demonstrating a strong reduction of immunogenicity in vivo [16]. In a dog renal transplant system, a significantly decreased cellular infiltration on posttransplant days 7–9 after PUVA therapy in comparison with untreated controls could be verified by means of fine-needle aspiration biopsy [8].

These results as well as the relatively simple and safe handling of PUVA therapy seemed to offer a new and effective approach to enhance kidney graft survival also in human beings. In 1987 we reported our first and encouraging results of an unrandomized open study concerning the efficacy of PUVA donor treatment in human kidney transplantation [17]. In comparison with a group of 26 non-pretreated kidneys, in the group of 33 PUVA-pretreated kidneys there were significantly fewer rejection episodes, fewer graft losses by irreversible rejections, fewer infections complications and an improved (but not significantly so) graft survival. Consequently, in 1989 we started a licensed, randomized, prospective, double-blind study in order to clarify the efficacy of PUVA therapy in human kidney transplantation. The aim of this paper is to present the final evaluation of the data of the 75 recipients included in that study.

Materials and methods

Study population

This study included 50 kidney donors. In a randomized manner, 25 of them received 8-methoxypsoralen (8-MOP) intravenously 10 min before starting the in situ perfusion. All kidneys were harvested by the Kidney Transplant Centre Berlin-Friedrichshain team between January 1989 and October 1990. During hypothermic preservation, the kidneys of 8-MOP-treated donors were UVA irradiated. A total of 75 kidneys was transplanted in the kidney transplant centers of Berlin, Halle and Rostock (Table 1), 14 kidneys were not suitable for transplantation, and 11 kidneys were sent abroad (Intertransplant and Eurotransplant organ sharing programmes). The complete data of these 75 recipients and the corresponding donors were available for this final evaluation. All patients were followed up for at least 24 months.

PUVA donor/graft treatment

8-MOP, obtained from Gerot Pharmazeutika (Vienna, Austria) as a 0.5% solution, was given intravenously at a dosage of 1 mg/kg body weight (b.w.) 10 min before initiation of in situ perfusion with Eurocollins solution ($n = 48$ donors) or University of Wisconsin (UW) solution ($n = 2$ donors). After en-bloc removal each kidney was flushed with cold Eurocollins of UW solution for 1 min. All kidneys were preserved by cold storage. As soon as possible (mean 3 h, range 70–280 min) after removal, the kidneys were irradiated with a 20 W mercury arc medium pressure lamp (UVS 20-2, NARVA, Berlin) for 4 h during hypothermic preservation at a distance of 29 cm. The UVA intensity during this time was measured as $1.3 \times 10^{-3}\ J \cdot cm^{-2} \cdot s^{-1}$.

Donor and recipient demographics

The pretransplant parameters of donors and recipients are summarized in Table 2. Concerning the distribution between PUVA and non-PUVA groups, there were no statistically significant differences.

Immunosuppression, rejection episodes, infections

The immunosuppressive protocols used are shown in Table 3. Most recipients (PUVA 28/36, non-PUVA 31/39) of both groups received initially a triple-drug therapy consisting of CsA, azathioprine and prednisolone. Only eight recipients in either group were initially treated with azathioprine and prednisolone ($n = 15$) or CsA and prednisolone ($n = 1$). About half of the recipients received induction therapy using rabbit anti-human T-lymphocyte globulin (ATG Fresenius, Oberursel, Germany). Details of these treatment protocols have recently been published [9]. For the diagnosis of rejection, clinical and laboratory signs as well as cytological and histological findings were decisive. The first line rejection therapy consisted of methylprednisolone (MP), 5 mg/kg b.w., for 3 to 5 days. In the case

Table 1 Transplantations were performed at the following centres

Kidney transplant centre	PUVA	Non-PUVA
Berlin-Friedrichshain/Charité	19	19
Halle	10	11
Rostock	7	9

Table 2 Donor and recipient demography

	PUVA	Non-PUVA
Donors		
Number	22[a]	25
Age (years)		
Mean ±SD	42±15	42±10
Range	11–64	24–60
Sex		
Female	10	13
Male	12	12
Cold ischaemia time (min)		
Mean ±SD	1142±362	1150±266
Range	360–2170	420–1745
Perfusion solution		
Eurocollins	20	25
University of Wisconsin	2	0
Preoperative CMV IgG status (ELISA)		
Donor/Recipient		
+/+	26	29
−/+	4	3
+/−	4	4
−/−	2	3
Recipients		
Number	36	39
Transplants		
Primary	33	34
Secondary	3	5
Sex		
Female	18	11
Male	18	28
Age		
Mean ±SD	40±12	40±12
Range	17–57	17–66
Presensitization (PRA ≧ 5%)		
Current	6 (16.7%)	6 (15.4%)
Peak	17 (47.2%)	18 (46.2%)
Renal disease		
Glomerulonephritis	22 (61.1%)	21 (53.8%)
Pyelonephritis	10 (27.8%)	8 (20.5%)
Polycystic kidney degeneration	1 (2.8%)	4 (10.3%)
Diabetes mellitus	1 (2.8%)	1 (2.6%)
Other	2 (5.6%)	5 (12.8%)

[a] The kidneys of three donors were sent abroad or were not suitable for transplantation. Therefore, their data are not included in this calculation

of MP-resistant rejections, ATG was used. Biopsy-proven vascular rejection was additionally treated with plasmaphereses or OKT3. Infections were classified as either major or minor. Major infections included pneumonia, sepsis, cytomegalovirus disease and invasive fungal infections. All recipients were followed up for at least 24 months. Differences between the groups were analysed using the chi-square test.

Results

Table 4 summarizes the early and late graft function as well as patient survival. The mean and median initial hospitalization times as well as the serum creatinine concentration upon hospital discharge were slightly better in the PUVA than in the non-PUVA group, but these differences were not statistically significant. After a follow-up of 24 months, both graft survival and patient survival were comparably high. One patient in each group died with a functioning graft. Table 5 presents the analysis

Table 4 Results

	PUVA	Non PUVA
Initial hospitalization time (d)		
Mean ±SD	37.6±16.0	44.0±34.1
Median	34	38
Early graft function		
Immediate	15 (47.7%)	28 (71.8%)
Delayed	17 (47.2%)	8 (20.5%)
Primary nonfunction	4 (11.1%)	3 (7.7%)
Discharged with functioning graft	30/36 (83.3%)	34/39 (87.2%)
Serum creatinine at discharge (μmol/ml)		
Mean ±SD	160±53	197±99
Graft survival		
3 months	29/36 (80.6%)	34/39 (87.2%)
6 months	29/36 (80.6%)	34/39 (87.2%)
12 months	28/36 (77.8%)	31/39 (79.5%)
24 months	27/36 (75.0%)	28/39 (71.8%)
Patient survival		
3 months	36/36 (100%)	39/39 (100%)
6 months	36/36 (100%)	39/39 (100%)
12 months	35/36 (97.0%)	39/39 (100%)
24 months	35/36 (97.0%)	38/39 (97.0%)

Table 3 Number of patients included in the various immunosuppressive protocols used (*CsA* cyclosporin A, *Aza* azathioprine, *Pred* prednisdone, *ATG* anti-human T-hymphocyte globulin)

Immunosuppressive protocols	PUVA		Non-PUVA	
	Initially	Third month	Initially	Third month
Triple: CsA/Aza/Pred	28	20	31	26
(with ATG inducation)	14/28	–	12/31	–
Double: CsA/−/Pred	0	7	1	8
(with ATG inducation)	0	–	1/1	–
−/Aza/Pred	8	2	7	0
(with ATG inducation)	3/8	–	3/7	–

Table 5 Analysis of complications

	PUVA	Non PUVA
Rejection episodes and infections		
a) Rejection		
Rate	25/36 (69%)	27/39 (69%)
No. of episodes		
0	11	12
1	19	19
2	5	6
3	1	2
Time of occurrence		
0– 3 month	4 (67%)	26 (67%)
> 3– 6 month	3	5
> 6–12 month	4	3
>12–24 month	1	1
b) Infections		
Sepsis	0	1
Pneumonia	3	1
CMV		
asymptomatic	7	4
mediocre	6	9
severe	1	1
Causes of graft loss		
Time of graft loss		
0– 3 month	7	5
> 3– 6 month	0	0
> 6–12 month	1[a]	3
>12–24 month	1	3[b,c]

Causes (PUVA)	Causes (Non PUVA)
Vasc. rejection inflammation	Acute vasc. rejection
Vasc. rejection prim non-function, thrombosis of V. renalis	Vascular rejection, prim. nonfunction
	Vascular rejection, prim. nonfunction
Severe vasc. and interstit. rejection, prim nonfunction	Acute rejection, prim. non-function
Graft arteriopathy, donor reactive antibodies, cell rejection, prim nonfunction	Chron vascular rejection, acute renal failure
Graft vasculopathy	Interstit. rejection
Vascular and cellular rejection	Chronic vasc. rejection and slight interstit. rejection
Chronic vascular rejection	Chronic vascular and interstit rejection
	Chronic vascular rejection

[a] Exitus letalis, cerebral bleedings, functioning graft
[b] Suizid, functioning graft
[c] Sepsis, no immunosuppression, ectomy of a swollen functioning graft

of all complications observed. There were no statistically significant differences in rejection rate, number and time of rejection episodes as well as infections complications. According to the causes of graft loss, PUVA therapy did not obviously influence humoral or vascular rejections. In all but one rejected and removed grafts, signs of vascular changes could be histologically demonstrated. In the non-PUVA group the proportion of chronic rejections was higher than in the PUVA group. Interestingly, the rate of late graft loss (6th to 24th month) was lower in the PUVA group than in the non-PUVA group (2 vs. 6 graft losses). Also, after subtraction of the two patients who died with a functioning graft and one removal of a functioning graft in a life-threatening situation, in the PUVA group there was only 1 late graft loss but in the non-PUVA group, 4.

Discussion

The UV region of the electromagnetic spectrum is arbitrarily divided into three regions: UVA (320–400 nm), UVB (290–320 nm) and UVC (200–290 nm). UVC has been termed germicidal radiation, but little of that radiation reaches the earth. UVA has minimal biological activity compared with UVB unless used in conjunction with a photosensitizer [10]. The combination of psoralen plus UVA radiation is known as PUVA therapy or photochemotherapy and is used in the treatment of cutaneous diseases including cutaneous graft versus host disease [3]. In the past few years the effect of PUVA therapy on the immune responses of experimental animals has received considerable attention. It has been shown that PUVA treatment inhibits HLA-DR antigen expression and the allogeneic mixed leukocyte reaction [5], alters the morphology and function of Langerhans cells [1, 19], reduces the MHC class II antigen expression in rat kidney and heart cells [2], causes local [6] and distant suppression of contact hypersensitivity [11], diminishes strongly kidney graft immunogenicity as shown in a retransplant model [16], induces humoral factors and cells able to transfer graft protection [16] and prolongs significantly the allograft survival time of murine skin [4], rat kidneys [13, 14] and rat hearts [18]. In addition, Von Gaudecker et al. [20] reported a significant down-regulation of donor-specific MHC class II molecules in all PUVA-treated kidney grafts. Five days after transplantation no donor-specific antigen-presenting cells (APCs) were seen in the graft, but increased numbers of recipient-specific cells identified as dendritic cells by morphological criteria were counted without any deleterious effect on the graft being noted. Thus, donor-specific dendritic cells have been replaced by APCs from the recipient, possibly mediating or characterizing a permanent acceptance of the graft. On the other hand, APCs from PUVA-treated as well as untreated kidney grafts were observed in the spleen. In contrast to APCs from untreated kidneys which include sensitization and clonal proliferation of host

lymphocytes, APCs from PUVA-treated kidneys lost their ability to present alloantigen to the recipient's lymphocytes: they became tolerogenic. Thus, during the first critical days the graft is protected from rejection.

These extended experimental experiences as well as the application of PUVA therapy in dermatology encouraged us to introduce this kind of immunoregulation into clinical kidney transplantation. In 1987 we reported on our first results of an unrandomized study. The number of rejection episodes was significantly lower in the PUVA group, and fewer grafts failed because of irreversible rejection (2 vs. 5). The graft survival rates at 12 months were 76% in the PUVA group and 65% in the non-PUVA group (NS). No negative side-effects were seen. In order to clarify the efficacy of PUVA therapy in human kidney transplantation, we consequently started a randomized, prospective, double-blind study in 1989. All donor kidneys were procured by the team of the Kidney Transplant Centre Berlin-Friedrichshain. The PUVA group as well as the non-PUVA group did not differ statistically with respect to the donor data (age, sex, cold ischaemia time, perfusion) or recipient data (age, sex, renal disease, first and second grafts, presensitization, immunosuppression, pretransplant CMV antobody status). We found no significant differences as to the initial hospitalization time and early graft function. Also, no variations were seen in the rejection rate, number and time of occurrence of rejection episodes as well as infectious complications. After a follow-up of 24 months, both graft survival (PUVA vs. non-PUVA: 75% vs. 71.8%) and patient survival (97.2%, 97.4%, respectively) were comparably high. The changed immunosuppressive protocols and the high rate of triple-drug-treated recipients have in total improved the graft survival rates, rendering a comparison with the 1987 results difficult. The 1-year graft survival rates in the non-PUVA groups were improved from 65% in 1987 to 79.5% in this study; in the PUVA groups such differences were not seen (1987 76%, at present 77.8%). Thus the overall improvement of graft survival in the CsA era weakens the effect of PUVA therapy in human kidney transplantation seen earlier in recipients treated only with azathioprine-prednisolone. The evaluation of complications shows quite clearly, that PUVA therapy did not influence humoral or vascular rejection. In all but one rejected and removed grafts, signs of vascular deterioration could be histologically determined. Therefore, the immunosuppressive protocols actually used are effective in preventing cellular or interstitial rejection, and humoral effectors appear to be much more responsible for acute graft loss as well as chronic rejection. Interestingly, the rate of late graft loss (6th to 24th month) was lower (but not statistically significantly so) in the PUVA group than in the non-PUVA group (2 vs. 6 graft losses). Thus, PUVA-pretreated kidneys could possibly have a benefit with respect to a reduced or delayed development of chronic irreversible rejection.

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