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Prolongation of Rat Renal Allograft Survival Time by Donor Pretreatment with 8-Methoxypsoralen and Longwave Ultraviolet Irradiation of the Graft (PUVA Therapy)

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Summary. Pretreatment of the kidney donor with 8-methoxypsoralen (8-MOP) and ex vivo longwave ultraviolet irradiation (UVA) of the kidney prolonged the subsequent survival on allogeneic recipients. The efficacy of this treatment seems to be dependent on the time and dose of UVA irradiation rather than on the dose of 8-MOP. In conclusion, PUVA treatment is effective in reducing the immunogenicity of the rat kidney allograft, although the mechanism remains unclear. These experimental findings are new and preliminary results in clinical human kidney transplantation are favourable.

Key words: PUVA, Kidney transplantation, Rat, UV irradiation.

Introduction

Exposure to nonionizing radiation in the form of ultraviolet light (UV) produces immunoaltering properties, including suppression of allergic contact dermatitis and delayed hypersensitivity, alteration of the antigenicity of molecules, impairment of the function of circulating lymphocyte subpopulations and induction of altered immune responses to cutaneous neoplasms [14]. Lindahl-Kiessling and Safwenberg [13] reported the effectiveness of UV irradiation in abrogating the stimulatory capacity of lymphocytes in an MLC, and Hayry and Andersson [7] showed that, although UV irradiated cells were unable to stimulate in primary cultures, these same cells can activate already primed T cells to cytotoxic activity. In a recent publication, Lau et al. [12] have shown that it is feasible to induce a state of tolerance by treating rats with UV irradiated blood cells of donor origin prior to grafting of pancreatic islet cells. Furthermore, the survival of full-thickness skin grafts in rabbits was prolonged by administration of 8-MOP and subsequent exposure of donor and recipient graft sites to UVA radiation [15]. Similar results were obtained by in vitro 8-MOP plus UVA (PUVA) treatment of allogeneic skin grafts in mice [4]. These and other findings [10, 18] suggested that in vitro T-cell proliferative response to alloantigens required antigen presentation by a metabolically active cell which was affected by UV irradiation or combined PUVA therapy. Theoretically, the application of PUVA therapy on kidney allografts also seems to be possible and useful.

The purpose of this paper is to report our studies using PUVA treatment of donor kidney allografts as a method of immunoalteration to prolong the kidney transplant survival in rats without immunosuppression.

Materials and Methods

Rats. The following inbred strains were used: BD IX and Sprague-Dawley (SD). These strains are different from each other in their major histocompatibility complex (MHC). The parental strains were bred at the Academy of Science of the GDR, Central Institute for Cancer Research, and hybrids from them were bred in the Experimental Animal House at this institution. All rats were maintained here.

According to our previous investigations [8] kidney transplantation was performed in the SD to BD IX and (BD IX × SD)F_1 to BD IX renal allograft model, in which passive enhancement provides incomplete suppression.

Kidney Transplantation. The technique used has been published in detail elsewhere [16]. Briefly, left orthotopic grafts with end-to-end anastomosis of the renal vessels and non-splinted end-to-end ureteric anastomosis were done using microsurgical technique. The right nephrectomy of the recipient was performed immediately after completion of the transplantation procedure. Graft function was followed by serial blood urea nitrogen (BUN) estimations (day 2, 5 or 7, 10, thereafter weekly). Autopsies were made on most rats that died and autopsy specimens were examined as reported previously [9].

Drugs. 8-MOP, obtained from GEROT Pharmazeutika (Vienna, Austria) as a 0.15% solution (Oxsoralen^R) was given i.v. at dosages of 0.06 and 1.0 mg/kg body weight (BW), respectively, via the vena cava of the kidney donor 10 min before removal of the kidney graft.

Table 1. Effect of PUVA therapy alone and combined with x-ray irradiation on graft survival of BD IX recipients of SD kidney allografts

Treatment group	No. of rats	BUN in mmol/l (mean ± SD) at days			survival times
		7	14	100	
1	10	76 ± 38	–	–	8, 8, 8, 9, 9, 9, 9, 10, 11, 11
2	10	76 ± 26	46 ± 18	12 ± 2	8, 8, 10, 10, 11, 14, 16, 53, > 100, > 100
3	10	49 ± 19	16 ± 12	10 ± 3	9, 9, 10, 13, 13, 20, > 100, > 100, > 100, > 100
4	10	58 ± 23	15	10	8, 9,,10, 10, 11, 11, 11, 12, 14, > 100
5	10	58 ± 29	27 ± 16	11 ± 4	9, 9, 10, 10, 10, 15, 16, > 100, > 100, > 100
6	10	46 ± 29	30 ± 12	12 ± 3	8, 8, 9, 10, 10, 10, 12, > 100, > 100, > 100
7	10	64 ± 26	29 ± 4	14 ± 1	8, 8, 9, 9, 10, 11, 12, 12, > 100, > 100

The renal toxicity of 8-MOP in SD rats was tested by administering 0.06 and 1.0 mg/kg BW in a single dose to 5 animals of each group. After 1, 2 and 12 weeks BUN level was estimated.

Ex Vivo Kidney Preservation and UVA Irradiation. Kidney transplants were placed in a micropuncture immediately after removal from the donor (University Manufactory, Uppsala, Sweden) with continuous kidney surface cooling at 8 °C using Euro-Collins solution. During this preservation the grafts were irradiated with a 40 W mercury arc medium pressure lamp (UVS 40-2, NARVA, Berlin, GDR) at a distance of 12.3 cm for 1 or 2 h. The UVA intensity was measured as 0.07 $J \cdot cm^{-2} \cdot s^{-1}$. After irradiation the grafts were transplanted.

Ex Vivo Kidney Preservation and X-Ray Irradiation. Immediately after removal from the donor the kidney was placed in a plastic bag containing Euro-Collins solution and melting ice and thereafter irradiated at 4.5 Gy (TUR, Transformatoren- und Röntgenwerk, Dresden, GDR). The time for x-ray irradiation was 5 min and 39 s and the total cold ischemic time ranged from 28 to 42 min, with a mean (± S.D.) of 37 (± 8) min.

Experimental Group Design.

Group 1: SD → BD IX: untreated control group
Group 2: SD → BD IX: 8-MOP 0.06 mg/kg BW, UVA 1 h
Group 3: SD → BD IX: 8-MOP 0.06 mg/kg BW, UVA 2 h
Group 4: SD → BD IX: UVA 2 h
Group 5: SD → BD IX: 8-MOP 0.06 mg/kg BW
Group 6: SD → BD IX: 8-MOP 1.0 mg/kg BW
Group 7: SD → BD IX: 8-MOP 1.0 mg/kg BW, x-ray 4.5 Gy
Group 8: (BD IX x SD)F_1 → BD IX: untreated control group
Group 9: (BD IX x SD)F_1 → BD IX: 8-MOP 0,06 mg/kg BW, UVA 2 h
Group 10: (BD IX x SD)F_1 → BD IX: 8-MOP 1,0 mg/kg BW

Statistical Analysis: The statistical evaluations were performed by means of the Chi-square test and Fisher exact test, and the differences among the groups were considered significant when $p < 0.05$.

Results

8-MOP Toxicity Experiment. 8-MOP at 0.06 and 1.0 mg/kg BW did not impair renal function as expressed by BUN level at any time of control.

Effect of PUVA Treatment Alone and Combined with X-Ray Irradiation on Graft Survival of BD IX Recipients of SD Kidney Allografts (Table 1). 8-MOP at 0.06 mg/kg BW and 1 h UVA irradiation (Group 2) did not improve renal function at day 7, but did increase graft survival. However, the difference is not significant ($p = 0.237$). Two rats survived more than 100 days. 8-MOP at the same dose and 2 h UVA irradiation (Group 3) modified the rejection markedly. The mean BUN level at day 7 was considerably, but not significantly, lower and survival times were increased significantly ($p < 0.05$), with 4 animals surviving for more than 100 days (Fig. 1). 2 h UVA irradiation alone (Group 4) and 8-MOP alone at 0.06 (Group 5) and 1.0 mg/kg BW (Group 6), respectively, did improve graft function at day 7 and increase graft survival, but fewer animals survived more than 100 days as in group 3. All differences were not significant ($p > 0.50$). In groups 5 and 6 (8-MOP alone) it should be noted that during transplantation the graft was exposed to UV light from the lamp of the operating microscope, but in a very low UVA intensity of 0.002 $J \cdot cm^{-2} \cdot s^{-1}$.

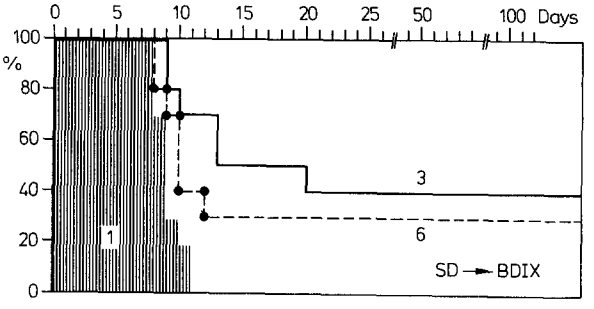

Fig. 1. Illustration of graft survival of 3 elected groups of BD IX recipients of SD kidney allografts (1 – group 1: untreated control, 3 – group 3: 8-MOP 0.06 mg/kg BW, UVA 2 h, 6 – group 6: 8-MOP 1.0 mg/kg BW)

8-MOP at 1,0 mg/kg BW plus x-ray irradiation (Group 7) did not improve renal function at day 7 and graft survival as compared with 8-MOP alone (Group 6; $p > 0.50$).

Effect of PUVA Treatment on Graft Survival of BD IX Recipients of (BD IX x SD)F_1 Hybrid Kidney Allografts (Table 2). After 8-MOP application at 0.06 mg/kg BW and 2 h UVA irradiation (Group 9) all but one animals survived

Table 2. Effect of PUVA treatment on graft survival of BD IX recipients of (BD IX x SD)F_1 hybrid kidney allografts

Treatment group	No. of rats	BUN in mmol/l (mean ± SD) at days			survival times
		7	14	100	
8	9	2,1 ± 0,7[a]	6,9 ± 0,9[a]	–	11, 11, 12, 12, 13, 14, 15, 15, 16
9	10	51 ± 31	18 ± 7	13 ± 4	11, > 100, > 100, > 100, > 100, > 100, > 100, > 100, > 100, > 100
10	10	66 ± 34	18 ± 14	12 ± 3	13, 18, 68, > 100, > 100, > 100, > 100, > 100 > 100, > 100

[a] Creatinine value (μmol/l)

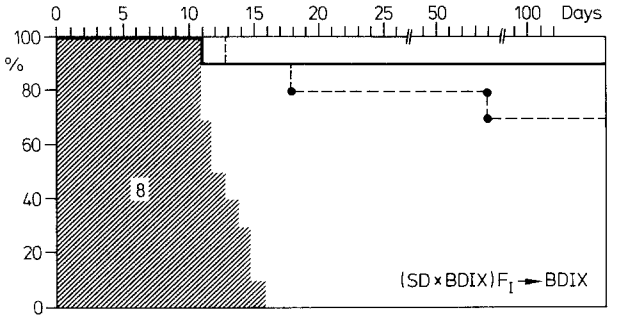

Fig. 2. Illustration of graft survival of BD IX recipients of (BD IX x SD)F_1 kidney allografts (8 – group 8: untreated control, 9 – group 9: 8-MOP 0.06 mg/kg BW, UVA 2 h, 10 – group 10: 8-MOP 1.0 mg/kg BW)

more than 100 days ($p < 0.001$) and BUN level returned to normal value. 8-MOP at 1.0 mg/kg BW alone (Group 10) did also increase graft survival significantly ($p < 0.01$). Seven out of 10 rats survived more than 100 days (Fig. 2). Likewise to group 5 and 6 in this group a restriction must be made regarding the UVA light emitted by the operating microscope lamp.

Discussion

Arguments for an immunosuppressive effect of PUVA come from transplantation studies in animals, experiments on allergic and irritative skin reactions, and clinical observations [17]. However, is UV light or PUVA application an immunosuppressive therapy?

In recent years, there has been a move away from the present reliance on recipient immunosuppression toward a greater emphasis on techniques that may be used to reduce the immunogenicity of the tissue being transplanted [11]. The concept that MHC antigens are not, by themselves, the barrier to successful allotransplantation is not new [5]. Hardy et al. [5, 12] extended these studies to show that these antigens must be presented on metabolically active antigen-presenting cells (Ia-positive macrophages or dendritic cells).

We have shown that PUVA treatment of rat kidney allografts prolongs the graft survival time significantly. The number of indefinitively surviving rats seems to be dependent rather on the time and dose of UVA irradiation than on the dose of 8-MOP. The best results were obtained by combined PUVA treatment as seen in group 3 and 9.

An explanation for this phenomenon is that these antigenpresenting cells are selectively damaged or inactivated by UV irradiation or PUVA treatment. It appears from the studies of Hardy et al. [5] that such inactivated cells, which carry unaltered MHC antigens, are effective in the induction of donor-specific tolerance in the adult animal, as shown by the indefinite survival of Lewis islet allografts in the ACI rat. Faustman et al. [2] suggested that such tolerance might be due to the action of suppressor cells. Recently, Fox et al. [3] suggested that antigen presented on UV-irradiated cells could generate suppressors. Furthermore, Faustman et al. [1] showed that elimination of Ia-positive cells from mouse islets permitted prolonged islet allograft survival in non-immunosuppressed hosts. However, such Ia-positive cells need not be eliminated physically, but may be simply inactivated [5].

Our findings which show that PUVA treatment prolongs kidney allograft survival are new. Because the kidney is a conglomerate of many cells with interspersed e.g. dendritic cells [6], the penetration of 8-MOP or UVA light may be incomplete; this would be explain the lack of 100% success in our experiments. The full acceptance by 90% of the recipients of PUVA-treated (BD IX x SD)F_1 kidney allografts clearly demonstrates that the kidneys have decreased immunogenicity after PUVA treatment without functional alteration. Although the mechanism of this tolerance is not fully understood, the existence of the phenomenon is one of the most encouraging recent developments in transplantation biology [11]. Preliminary results of application of PUVA treatment in human kidney allotransplantation have been favourable and will be published with further experimental data in near future.

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