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Synergistic Effect of Donor Pretreatment with 8-Methoxypsoralen and Ultraviolet Irradiation of the Graft Plus Azathioprine and Prednisolone Therapy in Prolonging Rat Renal Allograft Survival

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Summary. Pretreatment of the kidney donor with 8-methoxy-psoralen (8-MOP) and direct longwave ultraviolet (UVA) irradiation of the kidney graft (PUVA therapy) significantly prolonged survival in allogeneic recipients. 40% of the recipients survived more than 100 days with normal transplant function. The addition of standard clinical immunosuppressive agents azathioprine and prednisolone (both at dosages of 15 mg/kg body weight/day for 21 days) to the PUVA therapy further improved graft survival rate, with a recipient survival rate of 62.5%. The two drugs alone were less effective in prolonging graft survival rate (28.5%). A synergistic effect of PUVA therapy and standard immunosuppressive treatment with azathioprine and prednisolone was demonstrated. This suggested a possible clinical application of this type of immunosuppression and immunoregulation.

Key words: Kidney transplantatioon, Rat, PUVA therapy. Ultraviolet irradiation, Azathioprine, Prednisolone.

Introduction

A recent paper [21] showed that PUVA treatment of rat kidney allografts significantly prolonged survival time. The number of indefinitely surviving recipients seemed to depend on the time and dose of UVA irradiation rather than on the dosage of 8-MOP. The best results were obtained by combined PUVA therapy.

Other work has shown that ultraviolet (UV) irradiation can induce suppressor T cells and suppress several immune reactions [4, 18, 19]. The combined use of peritransplant clinical immunosuppressive agent cyclosporin A for 3 days has a marked synergistic effect in prolonging UV irradiated islet allograft survival compared to controls treated only with cyclosporin A [15]. In human cadaveric renal allotransplantation, the two other standard immunosuppressive drugs are azathioprine and prednisolone. Studies of the effect of these agents and combined PUVA treatment have not been reported. Since any clinical trial of PUVA therapy seems to be unlikely to be attempted without the concurrent use of azathioprine and prednisolone, this study was designed to show either a synergistic or an antagonistic effect of these drugs with PUVA therapy.

Material and Methods

Rats. We used the following inbred strains: BD IX and Sprague-Dawley (SD). These strains are different from each other at their major histocompatibility complex (MHC) and were bred at the Academy of Sciences of the GDR, Central Institute for Cancer Research. All rats were maintained in the Experimental Animal House of our institution. According to our previous studies [10] kidney transplantation was performed in the SD to BD IX renal allograft model, in which passive enhancement provides incomplete suppression.

Kidney Transplantation. The microsurgical orthotopic technique used has been published in this journal [20]. The renal vessels and the ureter of the left kidney were anastomosed end-to-end without use of an indwelling ureteral cannula. Right recipient nephrectomy was performed at the time of transplantation. Ischemia time ranged from 14 to 24 (mean 19) min. Graft function was followed by serial blood urea nitrogen (BUN) estimations (day 2, 7, 10, thereafter weekly). Blood samples were drawn from the medial angle of the eye. Autopsies were performed on most rats and which were examined as reported previously [11].

Lymphocytotoxic Antibodies. The determination of complement-dependent lymphocytotoxic antibodies was done by means of the ^{51}Cr-release test in Group 1 and 4. A pooled rabbit serum (dilution 1:10) served as a source of complement. Splenic lymphocytes isolated by centrifugation on a Ficoll-Visotrast-gradient (density: 1.078 g/ml) were used as target cells [5, 9].

Drugs. 8-MOP, obtained from GEROT Pharmazeutika (Vienna, Austria) as a 0.15% solution (Oxsoralen^{R}), was given intravenously (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 graft. Azathioprine for injection (Burroughs Wellcome, England) and prednisolone for injection (Jenapharm, Jena, GDR) were freshly prepared prior to application in sterile water and normal saline,

Table 1. Effect of PUVA therapy alone or azathioprine and prednisolone therapy alone, respectively, and combined PUVA plus azathioprine and prednisolone treatment on graft survival of BD IX recipients of SD kidney allografts

Experimental group	No. of rats	Survival times (days)	Comparison of survival times
1	10	8, 8, 8, 9, 9, 9, 9, 10, 11, 11	–
2	7	8, 8, 8, 9, 10, > 100, > 100	n.s.[a] vs. group 1 ($p = 0.15$)
3	10	9, 9, 10, 13, 13, 20, > 100, > 100, > 100, > 100	$p < 0.05$ vs. group 1 n.s. vs. group 2 ($p > 0.20$)
4	8	10, 20, 27, > 100, > 100, > 100, > 100, > 100	$p < 0.01$ vs. group 1 n.s. vs. group 2 ($p > 0.10$) n.s. vs. group 3 ($p > 0.30$)

[a] not significant

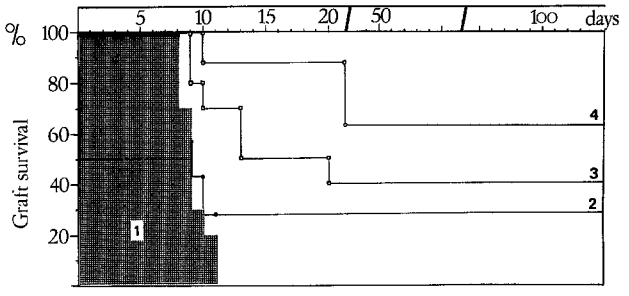

Fig. 1. Survival of rat renal allografts in the 4 experimental groups (see material and methods)

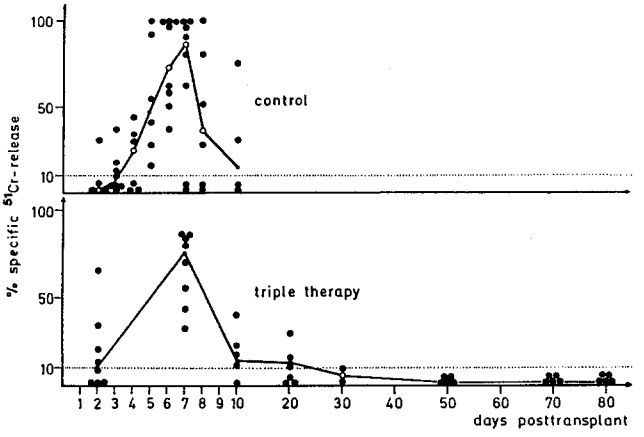

Fig. 2. Development of lymphocytotoxic antibodies after allotransplantation in group 1 (control) and group 4 (triple therapy) measured by ^{51}Cr-release assay. The black circles represent the individual values, the black line and the open circles the median values

respectively. Winearls et al. [23] suggested a less toxic dosage of azathioprine at 15 mg/kg BW/d and prednisolone at the same dosage, given for 21 days, which were used in this study. The dosage was selected on the basis of comparison with levels used in humans in the early posttransplant period, adjusted for surface area. On this basis the human dose of azathioprine of 3 mg/kg BW/d is equivalent to 15 mg/kg BW/d in the rat. The first injection was given i.v. at the time of transplantation, but all subsequent injections were given by the intraperitoneal route.

Ex Vivo Kidney Preservation and UVA Irradiation. The technique used has been described in detail in a recent paper [21]. The time of irradiation was 2 h and the UVA intensity was measured as 0.07 $J \cdot cm^{-2} \cdot s^{-1}$.

Experimental Groups. Group 1 ($n = 10$): no treatment. Group 2 ($n = 7$): azathioprine + prednisolone (both at 15 mg/kg BW/d for 21 days. Group 3 ($n = 10$): 8-MOP at 0.06 mg/kg BW, UVA 2 h. Group 4 ($n = 8$): azathioprine + prednisolone as in group 2, 8-MOP at 1.0 mg/kg BW, UVA 2 h.

Statistical Analysis. Comparison between the groups was made using Fisher's exact test or the Chi-square test. The differences among the groups were considered significant when $p < 0.05$.

Results

The results are summarized in Table 1. Control kidney grafts without treatment (group 1) were rejected rapidly. Azathioprine and prednisolone treatment alone (group 2) resulted in a prolonged graft survival and 2 out of 7 recipients (28.5%) survived more than 100 days. However, the difference is not significant compared with group 1 ($p = 0.15$). The PUVA therapy alone (group 3) did increase graft survival significantly ($p < 0.05$) compared with group 1. 40% of the animals survived more than 100 days with normal functioning allograft (Fig. 1).

The use of posttransplant azathioprine and prednisolone therapy has had a marked effect in prolonging the survival of PUVA-treated kidney allografts (group 4) and 62.5% of the recipients survived more than 100 days. This allograft survival rate was significantly better compared to the untreated control group 1 ($p < 0.01$), but the differences were not significant in comparison with group 2 and 3, respectively.

In all long-term surviving recipients of group 2, 3 and 4 ($n = 11$) the graft function remained stable, as judged by BUN levels, until the animals were killed (136th to 182nd day after transplantation).

The lymphocytotoxic antibody response after allotransplantation, with a peak on day 7 in both groups, was only slightly – if at all – suppressed in the combined therapy

group 4 (Fig. 2) in comparison with the control group 1. After the 20th day the median value was continuously below 10%.

Discussion

In our initial investigations [21] we found that PUVA treatment of rat kidney allografts significantly prolonged survival without the use of immunosuppressive drugs. The experimental data presented show that the temporary posttransplant application of azathioprine and prednisolone has a synergistic effect in prolonging survival of PUVA-preatreated rat kidney allografts compared with recipients treated only with azathioprine and prednisolone and those recipients with exclusively PUVA-pretreated allografts, respectively.

Azathioprine was found to have little effect on acute rejection [23]. This finding is consistent with the hypothesis that early rejection is primarily antibody mediated, since there was no difference in antibody response between control animals and animals treated with azathioprine. Further experiments [23] failed to show any significant synergistic effect between azathioprine and prednisolone and passive enhancement. Our results in group 2 which show little effect of azathioprine and prednisolone treatment in prolonging kidney allograft survival are consistent with these findings. However, a careful study on the effect of UV irradiation alone, PUVA therapy or combined PUVA plus azathioprine and prednisolone treatment on kidney allograft survival has not been reported. Although Lau et al. [14] have shown the possibility of tolerance induction by treating rats with UV-irradiated donor blood cells prior to grafting of pancreatic islet cells. In the same model longterm survival was obtained by direct UV irradiation of the donor tissue [6–8, 16]. It has been suggested that metabolically active antigen-presenting cells – APCs – (Ia-positive macrophages or dendritic cells) are inactivated by the use of UV irradiation [8]. Murine pancreatic islets pretreated with anti-dendritic cell antibody and complement prior to transplantation survived in histoincompatible recipients for more than 200 days. Rejection of stable islet allografts promptly occurred when transplant recipients were challenged with 1×10^5 donor dendritic cells 60 days after transplantation [3]. However, when transplanted animals were injected with UV-killed donor spleen cells, a source of alloantigen on non-stimulating cells, at 30 days posttransplantation, graft rejection was not stimulated. That means, the UV-irradiated cells were not immunogenic [12]. When animals which received UV-irradiated cells were subsequently challenged with viable donor spleen cells, the islet allografts were not rejected [13]. Faustman et al. [2] suggested that such unresponsiveness may result from the action of suppressor cells. The specifity of this tolerance was demonstrated by Lafferty and Prowse [12] as well as Lau et al. [14]. Lymphocyte from "tolerant" animals responded normally to donor alloantigen in vitro [1].

Pretreatment of rat kidney donor with 300 mg/kg cyclophosphamide on day –5 in addition with 1,000 rad total body irradiation on day –2 which depleted nearly all interstitial dendritic cells, produced a dramatic prolongation in survival of DA recipients of AS kidneys. Graft function remained stable in all animals with few signs of rejection in the seven-day biopsies [17]. In the same way, the application of donor-specific alloantibodies raised against nylon adherent spleen lymphocytes significantly prolonged the survival time of F_1 (BD IX x SD) kidneys in BD IX recipients [10].

These results in the kidney and pancreatic islet allograft model demonstrate an important in vivo role for donor dendritic cells in the stimulation of allograft rejection [3]. Our previous findings [21] that PUVA pretreatment prolonged kidney allograft survival could be explained by the alteration of those APCs. We have now shown a synergistic effect of PUVA pretreatment and posttransplant immunosuppressive therapy with azathioprine and prednisolone, but without 100% success. Despite this, attempts to remove or deplete cells with a great potential to stimulate a dangerous anti-graft response may be of some clinical value. On the other hand, the induction of graft protecting effectors (suppressor cells and/or enhancing antibodies) by the PUVA-treated donor tissue seems to be an other or additional mechanism involved in this phenomenon. Preliminary data [22] with UVA- and PUVA-treated donor-specific blood transfusions (DST) prior to kidney allotransplantation support this concept. The value of combined PUVA pretreatment and cyclosporin A application is under investigation.

We think that these 3 approaches including PUVA donor kidney pretreatment, PUVA-treated DST, and combined PUVA pretreatment with following (temporary?) conventional immunosuppressive therapy (azathioprine, prednisolone, cyclosporin A) are readily applicable to transplantation of kidney allografts in other animals, and eventually in humans. However, it should be emphasized, that all of these systems show strain dependencies, and further work is required to clarify the mechanisms responsible for indefinite graft survival.

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