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Inhibition of rat heart allograft rejection by a PUVA treatment of the graft recipient

Role of cisurocanic acid

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Abstract. Treatment of rat heart grafts with PUVA, the combination of the photosensitizer 8-methoxypsoralen and longwave ultraviolet light, leads to a prolonged transplant survival in allogeneic recipients. A PUVA treatment of the recipient rats, performed for 7 consecutive days after transplantation, prolonged graft survival even more effectively. This may be due to the systemic immunomodulatory effects of PUVA in the recipient. One of the mediators is urocanic acid, which is transformed by ultraviolet light in the skin from its trans- to the cis-isomer, which, in turn, acts as a mediator on the immune system. An injection of cisurocanic acid into graft recipients for 7 consecutive days after transplantation resulted in prolonged graft survival; in 40% of the rats, permanent graft acceptance was observed. The significance of these results for clinical organ transplantation is discussed.

Key words: Heart transplantation, rat, immune modulation – Immune modulation, heart transplantation, experimental – UV light in heart transplantation, experimental

In recent works, we and other investigators were able to show that treatment of grafts with ultraviolet light and especially with PUVA, the combination of the photosensitizer 8-methoxypsoralen (8-MOP) and longwave ultraviolet radiation (UVA, 320–400 nm), leads to an inhibition of their rejection after subsequent transplantation to allogeneic, otherwise untreated, recipients [4, 10, 13]. PUVA-treated murine skin grafts were rejected in a delayed fashion after allotransplantation [4], but rat kidney grafts were even accepted permanently in a high percentage of cases, depending on the strain barrier used [13]. Irradiation of rat pancreatic allografts with UVB light (290–320 nm) also resulted in permanent graft acceptance [8]. In a clinical trial of PUVA graft treatment in human cadaveric kidney transplantation, we achieved improved graft survival and a significantly lowered frequency of rejection episodes [14]. The mechanism by which a PUVA treatment of grafts inhibits their rejection can be explained only hypothetically by an inactivation of antigen presenting cells in the graft, which play an important role in the induction of the rejection response [4, 13].

Recently, Granstein et al. [3] showed that a PUVA treatment of mice, which were grafted with allogeneic skin, also prolongs graft survival. This may be brought about by systemic effects of a PUVA treatment on the immune system, effects which are due to the induction of T-suppressor cells [6, 7], redistribution of antigen-presenting cells to the irradiated sites [5] or to soluble mediators like prostaglandins [1], acute phase proteins [9], or as yet undefined cytokines [17]. One of the most likely mechanisms behind the systemic effects of ultraviolet light in the organism is urocanic acid (UCA), a constituent of the skin that is transformed by ultraviolet light from its trans- to the cis-isomer and that exerts a systemic immunosuppressive effect [2, 11]. The injection of cis-UCA, but not trans-UCA, has led to a suppression of contact sensitization in vivo [16] and of antigen presentation by epidermal cells in vitro [11, 15]. In this work we compare the effects of a PUVA treatment of the graft with those of a PUVA treatment of the recipient on rat heart allograft survival. In addition, we investigate the role of cis-UCA in this process.

Materials and methods

Animals

Inbred male rats (310–440 g) were obtained from the animal breeding center of the Institute for Cancer Research of the Academy of Sciences of the GDR at Berlin. Sprague Dawley/Bln rats (SD, RT-1^u) served as heart donors and BD IX/Bln rats (RT-1^d) acted as recipients. The animals were 14–20 weeks old at the start of the experiments and were kept in autoclaved cages in groups of maximally three animals per cage. The rats had free access to tap water and standard rat diet pellets (VDT R 13, KFM Biesenthal, GDR). Cages and bottles were changed and sterilized two times per week. The rats

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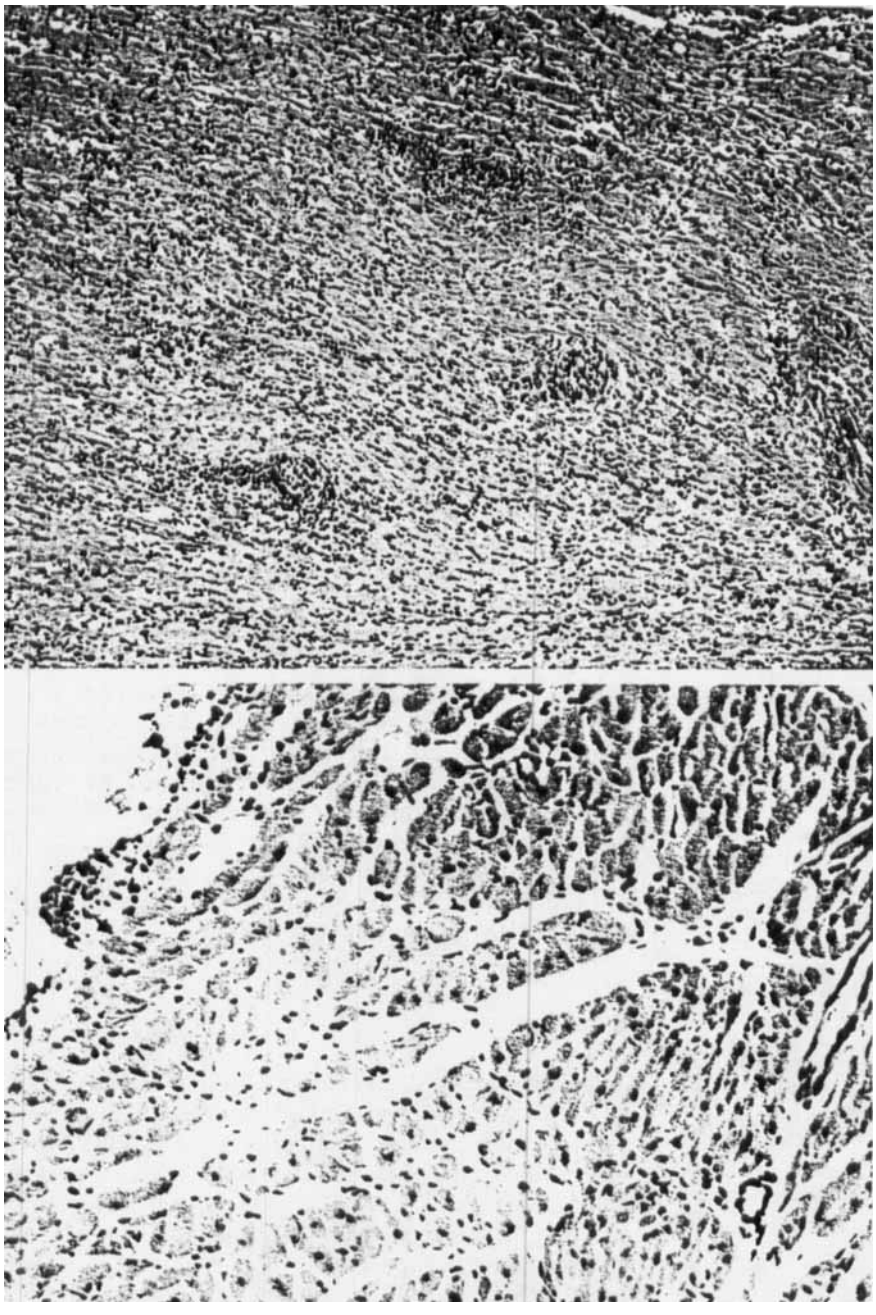

Fig. 1. Heart allograft from the untreated control group showing interstitial and vascular rejection (H & E, × 94)

Fig. 2. Heart allograft from the PUVA group showing a slight decrease in cell content but no signs of rejection (H & E, × 187)

were housed in a facility with a 12 h light/dark cycle at a constant temperature (24 ° ± 1 °C) and humidity (60% ± 10%).

Heart transplantation

The microsurgical donor preparation and transplantation technique have been published elsewhere [12]. Briefly, the donor heart was transplanted heterotopically into the unilaterally nephrectomized recipient. The brachiocephalic artery was anastomosed to the left renal artery with ten single sutures, and the pulmonary artery was anastomosed to the renal vein with two continuous semicircular sutures that were left untied. Both anastomoses were done end-to-end using 10-0 Ethicon (Ethicon GmbH, Hamburg, FRG).

Following closure of the abdomen, the animals were allowed to recover from anesthesia and were kept under the same conditions as preoperatively. The size and action of the grafted heart were assessed daily by palpation. Rejection was considered as the time of complete cessation of myocardial contractions, confirmed by histological examination, using hematoxylin eosin-stained sections. At the end of the experiment (cardiac arrest of function for more than 100 days), the animals were killed and their hearts removed for histopathological examination.

PUVA treatment

The photosensitizer 8-MOP, obtained as a 0.15% solution (Geroxsalen, GEROT Pharmazeutika, Vienna, Austria) was injected intraperitoneally at a dosage of 1 mg/kg body weight. The dorsal skin of the rats, shaved after transplantation with electric clippers over an area of 3 × 4 cm, was irradiated 30 min later under two parallel fluorescent lamps (UVS 40-2, Narva, Brand-Erbisdorf, GDR) emitting UVA light (less than 1% UVB light) at a distance of 20 cm for 5 min

Table 1. Graft survival after PUVA treatment

Experimental group and treatment	*n*	Graft survival (days)	*P*
1 None	9	5, 5, 6, 6, 6, 6, 6, 6, 10	–
2 PUVA-D[a]	10	7, 7, 8, 9, 10, 11, 18, 25, > 100, > 100	< 0.01[c]
3 PUVA-R[b]	10	7, 19, 22, 23, 65, > 100, > 100, > 100, > 100, > 100	< 0.05[d]

[a] Donor and graft
[b] Recipient
[c] Group 2 vs group 1
[d] Group 3 vs group 2

Table 2. Graft survival after UCA treatment

Experimental group and treatment	*n*	Graft survival (days)	*P*
1 None	9	5, 5, 6, 6, 6, 6, 6, 6, 10	–
2 UCA	10	11, 15, 17, 21, 22, 31, > 100, > 100, > 100, > 100	< 0.01

(radiation dose 6.35 J/cm^2). During irradiation each rat was immobilized in a plastic box.

A PUVA treatment of the graft was performed by an intravenous injection of 8-MOP (Oxsoralen) in a dose of 1 mg/kg into the donor rats, which were anesthetized 10 min thereafter. The hearts were removed quickly and irradiated with the same UVA source at a distance of 20 cm for 2 h. During the irradiation (radiation dose 8.4 J/cm^2), the hearts were stored in chilled Eurocollins solution and transplanted thereafter.

Urocanic acid treatment

Urocanic acid (Sigma, St. Louis, Mo) was dissolved in dimethyl-sulfoxide (5 mg/ml). This solution was dissolved tenfold with saline and irradiated for 1 h under a fluorescent sunlamp (FS 20, Westinghouse, Exeter, NH) emitting UVB light with an intensity of 3.5 J/cm^2. Thereafter, the solution, containing approximately 75% cisurocanic acid, was injected in 0.5 ml aliquots intraperitoneally into the recipient rats (dose 0.8 mg/kg).

Statistics

The nonparametric Mann-Whitney test was used.

Results

Table 1 shows that untreated rat heart allografts (group 1) were rejected soon after transplantation (mean survival time 6.2 ± 1.5 days). When the grafts were treated with PUVA (injection of 8-MOP into the donor and irradiation of the grafts with 8.4 J/cm^2 UVA light in vitro), 20% of them were accepted permanently (group 2); the remaining hearts were rejected in a delayed fashion ($P < 0.01$). A lower UVA dose (4.2 J/cm^2) led to only a moderate prolongation of graft survival (mean survival time 10.2 ± 8.7 days; data not shown in Table 1). A PUVA treatment of the recipient rats, performed for 7 consecutive days after transplantation, led to a permanent acceptance for more than 100 days in 50% of the recipients (group 3). In the other animals, graft rejection was delayed ($P < 0.05$). The doses of 8-MOP and UVA light used did not lead to sunburn reactions at the irradiated skin sites. No other side effects of the PUVA treatment were observed. Histological examination of the heart grafts confirmed these findings. Heart allografts, which were rejected in untreated recipients, showed massive cellular infiltration and typical signs of interstitial and vascular rejection (Fig. 1). In heart grafts that had survived in PUVA-treated recipients for more than 100 days, no signs of rejection, and only a slight decrease in cell content, were found (Fig. 2).

To test the role of the isomerization of trans-UCA to cis-UCA in the inhibitory effect of PUVA treatment on graft rejection, the recipient rats were injected for 7 consecutive days after transplantation of heart allografts with 0.8 mg/kg UCA, which had previously been irradiated with 1.2 J/cm^2 UVB light. This procedure has been shown to isomerize approximately 75% of the UCA to the cis-form [16]. The results depicted in Table 2 demonstrate that in 40% of the recipients, permanent graft acceptance (> 100 days) took place; the other animals rejected in a delayed form ($P < 0.01$). No toxic side effects of this treatment were observed. Histological examination of long-surviving heart grafts in cis-UCA-treated recipients showed no signs of rejection and very few cellular infiltrates. Treatment of the graft recipients with trans-UCA (unirradiated UCA) had no significant influence on graft survival (data not shown).

Discussion

The experiments described in this paper show that a PUVA treatment of rat heart allograft recipients prevents graft rejection even more effectively than a PUVA treatment of the graft, since a higher incidence of permanent graft acceptance (50% vs 20%) was observed. We propose that this graft survival prolongation effect is a result of the systemic immunosuppressive effects of ultraviolet light and PUVA [2, 6, 7, 11]. Histological examination of the grafts showed no signs of rejection in hearts surviving permanently (> 100 days) in PUVA-treated recipients. In contrast, typical signs of vascular and interstitial rejection were observed in heart grafts rejected in untreated recipients.

One of the soluble mediators induced by such a phototherapeutic treatment – cis-UCA – was used to mimic the effects of a PUVA treatment. A 7-day course of injection of cis-UCA into rat heart allograft recipients led to delayed graft rejection and permanent acceptance in 40% of the recipients. The lower incidence of permanent allograft acceptance in cis-UCA-treated rats than in PUVA-treated recipients may suggest that cis-UCA is not the sole mediator of a PUVA therapy in the organism. Other mechanisms and mediators may become active, among them induction of suppressor cells, lymphocytes, redistribution of antigen-presenting cells, release of prostaglandins, and acute phase proteins. In our current ex-

periments, we study the influence of most of these isolated mediators on heart allograft rejection in the rat.

The experiments described here further substantiate the systemic immunomodulatory effects of a photochemical treatment in the organism. Both a therapy of the graft recipient with PUVA and/or cis-UCA may gain clinical use to prevent transplant rejection.

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