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Effects of photochemical donor pretreatment on the pattern of dog renal allograft infiltrating cells

J. Kaden[1], H. Oesterwitz[2] and R. Schirrow[1]

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

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Summary. Kidneys were removed ten minutes after treating the donor with 1 mg of 8-methoxypsoralen per 1 kg b.w., during hypothermic preservation UVA-irradiated (intensity: 1.34 mJ/s) for 4 h and thereafter transplanted into ASDI dogs ($n = 10$). Fine needle aspiration biopsies were performed to analyze the inflammatory response. In comparison with untreated ASDI dogs ($n = 9$) PUVA pretreated renal grafts showed three important differences: 1. At the peak of the rejection process in the control group (day 8) the cellular infiltrate in the PUVA-pretreated kidneys was significantly diminished. 2. This reduction was caused by a significantly lower influx of monocytes/macrophages. 3. The peak of the cellular response in the PUVA group was delayed. These findings support the concept of reduced graft immunogenicity by pretransplant PUVA treatment.

Key words: Dog renal transplantation – Ultraviolet radiation – Donor pretreatment – PUVA – Fine needle aspiration biopsy – Rejection

Introduction

At present the reduction of graft immunogenicity prior to transplantation seems to be the method of choice for a prolonged graft survival thus avoiding additional alteration of the host's immune system. The transfer of such experimental conditions into the clinical situation might be possible. We have demonstrated the prolongation of rat kidney [11–14] and heart [15] graft survival times by treating the donor with the photosensitizer 8-methoxypsoralen (8-MOP) and by a subsequent pretransplant in-vitro irradiation of the organs with longwave ultraviolet light (UVA). This kind of pretreatment also resulted in a reduction of MHC class II antigen expression on rat kidney and heart cells [6]. This could explain the positive effect on the survival time.

Before transferring this treatment protocol into clinical practice we decided to verify the effects observed in the rat model in a similar dog renal transplant model. We have already published the details of the results obtained [16]. In this paper we show the distinct influence of PUVA therapy on the pattern of graft infiltrating cells examined by fine needle aspiration biopsy in comparison to untreated dog kidney graft.

Material and methods

Animals

Male and female dogs of the ASDI race were used (breeder: VOK Versuchstierzuchtobjekt Gross Boernicke, GDR) aged between 6–11 months with body weight of 10.8–19.9 kg.

Donors and recipients were mixed lymphocyte culture and sex-matched siblings. The PUVA group consisted of 10 and the control group of 9 dogs.

PUVA treatment

Ten minutes before removing the kidneys the donors received 1 mg 8-MOP/kg b.w. (OXORALEN, Gerot Pharmazeutika, Vienna, Austria) intravenously. Immediately after removal the kidneys were flushed for 1 min with cold Euro-Collins solution. Then the kidneys were irradiated with a 20 W mercury arc medium pressure lamp (UVS 20-2, VEB NARVA Berlin, GDR) for 4 h during hypothermic preservation (distance 29 cm, UVA intensity: 1.34 mJ/s).

Kidney transplantation

Details of the technique have been published [16]. The kidney was heterotopically grafted into the right or left femoral region of the recipient. The A. renalis was anastomosed end-to-end to the A. femoralis superficialis, the renal vein was anastomosed end-to-side to the femoral vein, and the ureter was anastomosed (antireflux) to

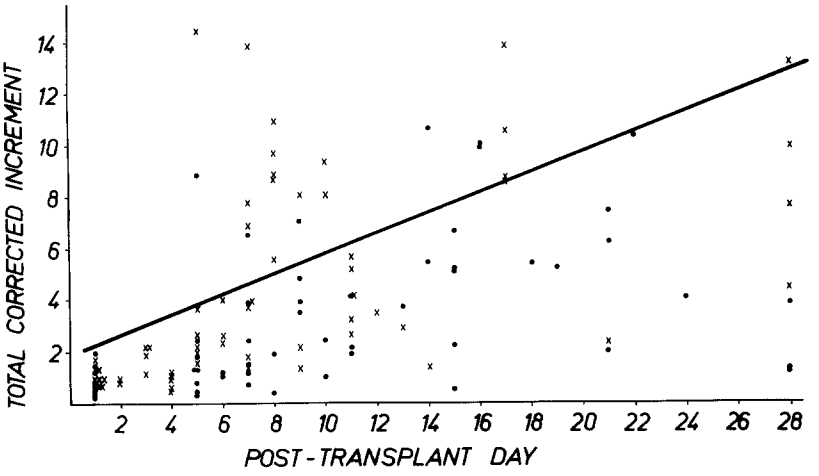

Fig. 1. Distribution of the Total Corrected Increments (TCIs) of allografted dog kidneys in the immediate posttransplant period. The asterisks represents the TCIs in the controls, the black points represent the TCIs in the PUVA group. The black line serves as a discrimination line and demonstrates the time-dependent increase of the TCIs as well as the differences of TCI peaks in terms of time and height. Note the rejection process between the 7th and 9th postoperative day in untreated dogs

the extraperitonalized bladder through a submucosal tunnel. Finally a bilateral nephrectomy of the recipient was performed. No additional immunosuppressive therapy was given.

Fine needle aspiration biopsy (FNAB)

FNAB was done every second or third postoperative day. In total, 124 graft biopsies were evaluated. Equipment, aspiration technique and the processing of the material followed the guidelines originally described by Häyry and von Willebrand [9]. 200 µl samples of suitable diluted aspirate and peripheral blood were cytocentrifuged (CYTOSPIN 2, Shandon, UK) onto precleaned microscopic slides. After air drying the specimes were stained with May-Grünwald-Giemsa and microscopically examined. In order to obtain the real cellular infiltration of the graft the difference between the percentage of white blood cell count in the aspirate and in the peripheral blood has to be calculated. This "increment" was converted into a "corrected increment" using correction factors for the different white cell types according to their known significance in the inflammatory process of rejection, e.g. the number of lymphoblasts, plasmablasts, plasma cells and macrophages were multiplied by 1.0, activated lymphocytes by 0.5, large granular lymphocytes and monocytes by 0.2, small lymphocytes and granulocytes by 0.1. The sum of these corrected increments (CIs) represents the "total corrected increment" (TCI), our scoring system.

Statistics

The statistical significance of our experiments was ascertained using Student's *t*-test and Chi-square test.

Results

Graft function

The serum creatinine level in the PUVA group on the 7th postoperative day was significantly lower than in the untreated control group (158 ± 22 vs. 280 ± 41 µmol/l; $P < 0.01$).

Transplant cytology

Analysis of inflammatory response in the untreated kidney Graft. Figure 1 shows all totaly corrected increments (TCIs) obtained within the first 21 postoperative days. All the TCIs were unchanged without any exeption until day 4, ranged from 0.5 to 2.2 and consisted mainly of polymorphonuclear granulocytes. At day 5 the TCIs gradually rose, and on days 5 and 6 the cellular infiltrate consisted of a mixture of eosinophils (0–12%), monocytes (0–12%) and small lymphocytes (3–32%), the ICIs ranged from 1.5 to 14.4. At day 7, monocytes became the predominant cell type, lymphocytes declined in number, but a few lymphoblasts (0–5%) could be seen. The "immunoactivation" was first diagnosed on day 6 ($\times \pm s = 6.5 \pm 1.1$) and TCIs ranged from 3.6 to 14.4 ($\times \pm s = 6.6 \pm 3.7$). TCIs peaked on day 8 ($\times \pm s = 7.8 \pm 1.5$) and ranged from 5.5 to 14.4 ($\times \pm s = 9.9 \pm 3$). Five of the nine control dogs died between day 8 and 12. In the other 4/9 dogs who survived for 24, 26, 26 and 60 days, the TCIs showed a second peak on days 17, 17, 24 and 27 ranging from 4.4 to 13.8. At that time the portion of lymphocytic cells was slightly higher than that of monocytes/macrophages (12–55% vs. 8–30%).

Inflammatory response in PUVA-treated kidney grafts. In order to compare the inflammatory responses in untreated and PUVA-treated kidney grafts, 3 time-intervals were chosen: postoperative days 1–3, 4–6 and 7–9. For statistical evaluation the TCIs of the intervals (1 value per dog and per interval) were separately added and compared. Figure 2 shows the added TCIs of both the control and the PUVA group. Until day 6 (2nd interval) no differences in the size of the inflammatory response was observed. However on postoperative days 7–9 the inflammatory response induced by PUVA-treated kidney grafts was significantly

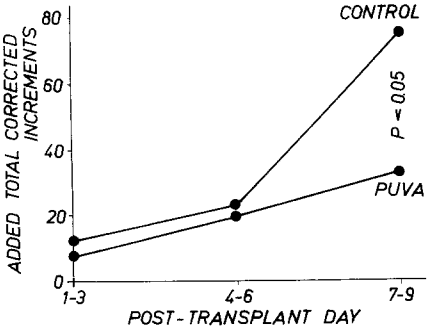

Fig. 2. Comparison of added TCIs at three definite postoperative intervals. Note the statistical significant difference of the TCIs at the 7th to 9th postoperative day of both groups

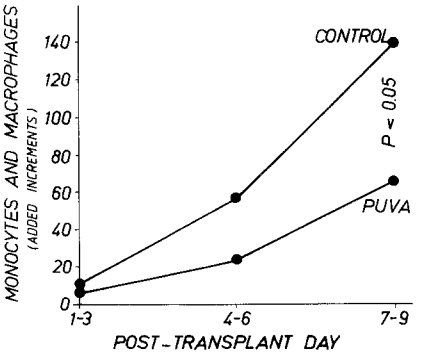

Fig. 3. Comparison of the added monocyte and macrophage increments at three definite postoperative intervals. At the peak of the rejection process in untreated dog kidneys the number of monocytes and macrophages in PUVA treated dog kidneys was significantly lower

$P<0.05$) lower than that of untreated grafts. This difference was solely caused by a significant ($P<0.05$) reduced content of monocytes and macrophages (Fig. 3). All other cell types were not quantitatively influenced by PUVA and showed a similar pattern to those in the control groups.

Comparing the single values of the TCIs (Fig. 1) it is obvious, that the TCIs of the PUVA group compared with the TCIs of the control group peaked lower and later. The black line in Fig. 1 demonstrates this shifting of the TCIs in the PUVA group from lower values during the first 2 postoperative weeks (in contrast to the control values, which were above the discriminating line, in particular between the days 7–9) to higher values in the following days. The typical and strong cellular infiltration indicating the rejection process between the 7th and 9th postoperative days in untreated kidney grafts was not observed in PUVA-treated kidney grafts.

Discussion

The pretreatment of both the graft donor and the graft itself might reduce graft immunogenicity. In 1974 Zincke and Woods [18] reported a significant reduction in the severity of the rejection response of canine allografts from donors pretreated with procarbazine hydrochloride, cyclophosphamide and methylprednisolone. Brom et al. [2] using cyclophosphamide and methylprednislone for donor pretreatment extended the survival time of mismatched Beagle renal allografts from 9 ± 1.8 to 17 ± 18 days. Beside the influence on passenger leukocytes the effect of this type of pretreatment is mediated by drugs within the graft at the time of surgery.

A new approach to enhance graft survival time was made by introducing photochemotherapy into organ transplantation. In 1985 we reported the prolongation of rat renal allograft survival time by PUVA therapy [11], which combines donor pretreatment with the photosensitizer 8-MOP and pretransplant in vitro UVA radiation of the graft. In both rat kidney and heart transplant experiments we confirmed the efficacy of this type of donor pretreatment [12–15]. Also in murine skin [8], heterotopic murine cornea [17] and rat pancreatic islet allograft models [10] a significant reduction of rejection by PUVA or UV radiation has been demonstrated.

According to the mode of action of photochemotherapy by means of immunohistological studies using monoclonal antibodies to rat MHC class I and class II antigens we showed a significant reduction of class II antigen expression in kidney and in heart cryostat sections after PUVA treatment [6]. This reduction of donor MHC class II antigens may influence both the antigen presentation and the activation of T helper cells and could therefore explain the effects observed. In 1984 Granstein et al. [7] reported on a decreased I-A antigen expression 24 hours after exposure of low density spleen cells to UV radiation (UVR). In a mixed skin cell-lymphocytes culture reaction the lymphocytes-stimulating ability of UV-irradiated epidermal cells was decreased [3]. This effect however was more the result of the transient inhibition of the antigen processing function of the Langerhans cells (LC) than of an alteration in membrane antigen expression. Cooper et al. [4] also described a direct effect of UV on the alloantigen-presenting function of LC, but accented the importance of timing and dosage of UV exposure as critical factors determining the effect on immune function.

In order to verify PUVA effects in another species we chose the dog allograft model and followed the inflammatory response in the graft by FNAB. In agreement with the findings of Block et al. [1] in

untreated controls, the transplant aspirate cytology (TAC) showed evidence of inflammation between the 5th and the 9th postoperative day. The cellular infiltrate initially consisted (day 5–6) of eosinophils, monocytes and mostly of lymphocytes. Thereafter monocytes became the predominant cell type and beginning on day 8 macrophages appeared. The number of lymphoblasts was always very low. On the same day TCI showed its first peak ranging from 5.5 to 14.4 (average 9.9). Interestingly, precursor cytotoxic T lymphocytes (CTLp) frequencies also increased after day 4 and reached a value 9 times higher in the kidney than in the peripheral blood on day 7 [5].

All animals surviving this first rejection episode (4/9) in the 3rd or 4th postoperative week experienced a second rejection episode characterized by a mixture of monocytes/macrophages and comparatively more lymphocytes. In connection with this second rejection episode all 4 animals died.

The analysis of the cellular infiltrate of PUVA pretreated kidney grafts showed three important differences from the untreated controls. Firstly, the inflammatory response induced by PUVA-pretreated renal allografts was significantly lower than in controls. This is probably the expression of the reduced graft immunogenicity induced by PUVA. Secondly, this reduced cellular infiltrate was caused only by a significantly reduced influx of monocytes and macrophages. Thirdly, the highest TCIs in the PUVA group occured later in the postoperative cource. Thus, PUVA treatment also resulted in a delayed and reduced inflammatory response in a much larger organ than that of a rat kidney. This effect as well as the simplicity of this pretreatment protocol and the absence of an additional influence on the recipient in our opinion justify its application in clinical kidney transplantation.

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Dr. sc. med. J. Kaden
Abteilung für Experimentelle
Organtransplantation
Leninallee 49
DDR-1017 Berlin
German Democratic Republic