The effect of duration and timing of systemic cyclosporine therapy on corneal allograft survival in a rat model

Abstract Background: Systemic cyclosporine A (CsA) remains a valuable treatment option in the prevention of corneal graft rejection, but the question of timing and duration of this systemic therapy remains unresolved. The effect of a pre- and postoperative dosing schedule, related to the expected moment of rejection, was examined in a rat model.

Methods: All AO (strain) recipients of PVG grafts were assigned to the following treatment groups: Group 1 (controls), groups 2–5 (a postoperative treatment regimen of CsA for 5, 10, 15 and 30 days respectively) and groups 6 and 7 (CsA preoperatively for 5 days and postoperatively for another 5 or 10 days respectively). Corneal allografts were clinically evaluated and blood CsA levels were measured at various time points.

Results: Untreated controls rejected their allografts after 13 days. Regression analysis showed a strongly significant positive correlation between graft survival time and duration of cyclosporine therapy. There was no difference in graft survival between groups 3 (CsA 10 days) and 4 (CsA 15 days). A pre-operative dosing schedule of CsA followed by postoperative treatment had no advantage over a solely postoperative treatment regimen. The moment of rejection was characterized by a low to undetectable CsA concentration.

Conclusion: The present study demonstrates a significant influence of the duration of systemic CsA administration on allograft survival time. However, preoperative administration of CsA does not seem to have an additional influence on graft survival, which is in line with the biological evidence of the mechanism of action of CsA on the effector arm of graft rejection.

Introduction

Corneal transplantation is one of the most successful forms of solid tissue allografting. There is, however, a substantial decrease in success rate after 5–10 years, especially in high-risk patients. Even nowadays, the leading cause of transplant failure remains allograft rejection. Hence, there is a continuing need for further studies with new and existing therapeutic agents that modulate the immunological mechanisms of corneal graft rejection [26].

Cyclosporine A (CsA) is a hydrophobic, cyclic endopeptide produced by the fungus *Tolypocladium in- flatum* Gams. CsA is a potent and selective inhibitor of lymphocyte function which has proven to be a clinically effective immunomodulator. Cyclosporine has been commercially available for systemic use since 1983. It has been extensively used for the prevention of graft rejection in solid organ and tissue transplants (including corneal allografts) and more recently for autoimmune diseases such as rheumatoid arthritis and severe plaque psoriasis. Possible indications for topical CsA in ophthalmology include Mooren’s ulcer [30], ligneous conjunctivitis [9], peripheral rheumatoid ulceration [14] and other ocular surface inflammatory disease [10].
The topical use of CsA in the prevention of corneal allograft rejection remains debatable: different experimental models show conflicting results [12, 17]. No large randomized double-blind clinical studies in patients have been performed. Therefore systemic CsA remains a valuable treatment option in the prevention of graft rejection, especially in high-risk patients. Since potentially severe side effects limit chronic administration, it is difficult to decide how long systemic CsA should be given after corneal transplantation. Systemic CsA does prolong corneal graft survival in experimental models while it is being administered [1, 2, 18]. The present study investigates the effect of the pre- and postoperative dosing schedule of systemic CsA on corneal allograft survival in a rat penetrating keratoplasty model, with regard to the expected moment of rejection.

Materials and methods

Animals

In all experiments, inbred male rats (Harlan Sprague-Dawley, Bicester, UK) weighing 200–250 g were used (minimum age 8 weeks). AO rats served as recipients of corneas from PVG rats. These strains differ in both minor and major histocompatibility complex antigens (respectively RT1u and RT1c) [5]. Animals were handled in accordance with the National Institute of Health “Guide for the Care and Use of Laboratory Animals”, and all experiments were approved by the Animal Care and Ethics Committee of Ghent University Hospital. Rats were housed under standard conditions, fed with a standard laboratory diet and given free access to tap water.

Surgical procedure

Orthotopic corneal transplantations were performed on the right eye of AO rats using the method described by Williams et al. [29], with some modifications (oversized trephine, running suture with a buried knot).

Donor animals were killed by an overdose of pentobarbital 60 mg/kg (Nembutal, Abbott, Louvain-la-Neuve, Belgium) and the corneal buttons were removed with a 3.5-mm trephine (Stieffel, Leuven, Belgium) and curved Vannas scissors. The donor cornea was stored in balanced salt solution (BSS; Alcon, Puurs, Belgium) at 4 °C until use (for a maximal duration of 30 min).

Prior to surgery, maximal mydriasis was induced in the recipient eye with two drops of 10% phenylephrine hydrochloride (Chauvin Pharmaceuticals, Essex, UK) in order to avoid surgically induced iris damage and anterior synechiae. Recipients were anesthetized with an intramuscular injection of a mixture of ketamine 90 mg/kg (Ketalar; Warner-Lambert Manufacturing, Parke-Davis, Zaventem, Belgium), xylazine 7.5 mg/kg (Rompun; Bayer, Brussels, Belgium) and atropine 0.1 mg/kg (Asta Medica, Brussels, Belgium). A 3.0-mm trephine (Storz, Bausch and Lomb Surgical, Antwerp, Belgium) was used to open the anterior chamber of the recipient eye, and a drop of visco-elastic substance (Healon, Pharmacia&Upjohn, Brussels, Belgium) was instilled.

The donor button was secured with 8–10 bites of a continuous 10.0 monofilament nylon suture (Alcon) placed intrastromally and tied into the wound with a triplicate knot cut flush to the surface. No attempt was made to reform the anterior chamber, since it spontaneously reformed within 24 h, and the sutures were left in place for the duration of the experiment. Immediately after grafting and on the first postoperative day an antibiotic ointment (Aureomycine, Asta Medica) was applied to the operated eye in both experimental and control animals.

Graft assessment

Grafts were examined daily using an operating microscope until postoperative day 14 (POD 14) and thereafter on alternate days until rejection occurred or for at least 70 days after grafting. Survival of a graft for more than 70 days was considered as ‘indefinite’. The transplants were evaluated by means of a scoring system which assessed three parameters (opacity, edema and neovascularization) on a scale of 0–4 [11]. The time of graft rejection was recorded as the day when the combined score reached 6 or more (26/12 years). This provided a clear distinction between rejected and non-rejected grafts, since the maximum score achieved in a syngeneically transplanted control group (AO to AO) was 3 (data not shown).

Experimental design

A total of 65 animals underwent orthotopic corneal transplantation. One was excluded on the first postoperative day because of technical failure (group 5). All AO recipients were arbitrarily assigned to one of seven treatment groups (Table 1). A first group consisted of 20 untreated allografts, which served as controls. The recipients of groups 2–5 were assigned to a postoperative treatment regimen of CsA for 5, 10, 15 and 30 days respectively. Groups 6 and 7 consisted of rats receiving CsA preoperatively for 5 days and postoperatively for another 5 or 10 days respectively.

Intramuscular injections of CsA (dose 10 mg/kg) were prepared from Sandimmune (intravenous CsA stock solution 250 mg/5 ml, a gift from Novartis Pharma, Brussels, Belgium) by diluting it with 0.9% normal saline. Injections were administered on the day of surgery and once daily during the time of treatment. The animals were weighed daily to adjust the CsA dosage to 10 mg/kg.

Histopathological findings

At the time of maximal rejection (a score of 6 or more for 3 days), the animals were killed with an overdose of pentobarbital. The grafted corneas were dissected, fixed with 10% neutral buffered formalin and embedded in paraffin. Five-micrometer-thick sections were stained with PAS and examined for histological evidence of rejection.

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<th>Group</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>1</td>
<td>Untreated allografts</td>
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<td>Controls</td>
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<td>2</td>
<td>CsA for 5 days</td>
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<td>CsA5</td>
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<td>3</td>
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<td>5</td>
<td>CsA for 30 days</td>
<td>9</td>
<td>CsA30</td>
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<td>6</td>
<td>CsA for 5 days pre- and 5 days postoperatively</td>
<td>5</td>
<td>CsA5–5</td>
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<td>7</td>
<td>CsA for 5 days pre- and 10 days postoperatively</td>
<td>10</td>
<td>CsA5–10</td>
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Table 1 Summary of the seven treatment groups