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ORIGINAL ARTICLE

Topical FK506 as immunoprophylaxis after allogeneic penetrating normal-risk keratoplasty: a randomized clinical pilot study

Thomas Reinhard,[1] Susanne Mayweg,[2] Alexander Reis[2] and Rainer Sundmacher[2]

1 LIONS Cornea Bank Regio/Baden-Württemberg and Eye Hospital, Albert-Ludwigs University, Freiburg, Germany
2 LIONS Cornea Bank North-Rhine Westfalia and Eye Hospital, Heinrich-Heine University, Düsseldorf, Germany

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Correspondence
Thomas Reinhard MD, LIONS Cornea Bank Regio/Baden-Württemberg and Eye Hospital, Albert-Ludwigs University, Killianstr. 5, 79106 Freiburg, Germany. Tel.: +49 761 270 4005; fax: +49 761 270 4063; e-mail: reinhard@aug.ukl.uni-freiburg.de

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Summary

The purpose of this study was to evaluate for the first time the efficacy and safety of topical FK506 in patients undergoing penetrating normal-risk keratoplasty in a prospectively randomized clinical trial. Twenty patients were treated with FK506 0.06% three times per day for 6 months postoperatively. An additional 20 patients received five drops of prednisolone acetate 1% tapered within 6 months. All patients received 1 mg/kg bodyweight/day of systemic fluocortolon tapered within 3 weeks postoperatively. Clear graft survival, ratio of immune reactions and side effects were the main outcome measures. One year postoperatively all patients of the FK 506 group were free from immune reactions, in contrast to 84% in the steroid group (Kaplan–Meier values; $P = 0.9$ in the log rank test). None of the patients developed irreversible graft failure so far. In eight patients of the FK506 group premature withdrawal of the drug was deemed appropriate because of local side effects. FK506 might turn out to become an effective immunoprophylaxis in subjects undergoing penetrating normal-risk keratoplasty. Local discomfort should be further reduced.

Introduction

More than 50 years after the first description of an immune reaction following penetrating keratoplasty [1], this complication still represents the major threat for the graft [2–5]. Topical steroids are an effective prophylaxis of immune reactions if administered in a dose of 3–5 drops per day [6]. As a result of severe side effects like steroid response glaucoma, cataract, surface disorders or infections such a prophylaxis is mostly limited to some months postoperatively [7]. Efficacy of topical cyclosporin A (CsA) has never been proven in prospectively randomized clinical trials [8–10]. Systemic immunosuppressives like CsA or mycophenolate mofetil improve graft prognosis, but their use may be associated with severe side effects like nephrotoxicity or hepatotoxicity. Therefore, they are only administered in high-risk situations [11–15].

FK506 is an antibiotic produced by *Streptomyces tsukubaensis* [16,17]. Like CsA it inhibits proliferation of T lymphocytes. FK506 blocks transcription of a variety of cytokines, especially of interleukin 2, in a similar manner as CsA [18–20]. The immunosuppressive potential of FK506 is 30–100 times higher than that of CsA [21,22]. Furthermore, topical FK506 penetrates more easily into the cornea [23,24].

After solid organ transplantation, systemic FK506 represents one of the systemic immunosuppressive standard prophylaxis in combination with systemic steroids and systemic azathioprin or systemic mycophenolate mofetil [25,26]. Topical FK506 is highly effective in the treatment of atopic dermatitis [27].

In the allogeneic rat, keratoplasty model efficacy of topical FK506 could be shown [28–30]. Recently, we presented data from a compassionate use trial indicating efficacy in patients with atopic blepharokeratoconjunctivitis,

Thygeson's superficial punctate keratitis and nummular adenoviral keratitis [31]. In penetrating keratoplasty topical FK506 has not been tested so far.

Here, preliminary results of a prospectively randomized clinical trial in which FK506 is being tested against a topical steroid are reported.

Patients and methods

Forty subjects undergoing normal-risk keratoplasty were included in the study after approval by the local ethics committee and after having properly obtained written informed consent. The study was performed according to the 1964 Declaration of Helsinki.

Study groups

As experience with this new topical immunomodulative drug is very limited, only 40 patients were included in this study [31]. Twenty patients were treated with topical FK506 three times per day for 6 months postoperatively (group I). Further 20 patients received five drops of prednisolone acetate 1% tapered within 6 months (our standard postoperative therapy, group II). After these 6 months, the patients of both study groups did not receive any immunosuppressive therapy.

FK506 eye-drops

FK506 was dissolved in polyvinyl alcohol, benzalkonium chloride, sodium chloride, sodium dihydrogenphosphate dihydrate, disodium hydrogenphosphate dodecahydrate, phosphoric acid, sodium hydroxide and sterile purified water. The concentration of FK506 was 0.06%.

Patient selection and definition of normal-risk keratoplasty

Normal-risk patients were selected in order to minimize the risk for nonimmunological failure by surface disorders (e.g. in limbal stem cell insufficiency), glaucoma or herpetic recurrences. This was performed because in some cases it may be difficult to differ between immunological and nonimmunological graft failure.

Only patients undergoing first keratoplasty within an avascular host cornea were included. All grafts with a diameter of 7.7 mm were positioned centrally. Indications for surgery were keratoconus, Fuchs' endothelial dystrophy, bullous keratopathy and nonherpetic scars (Tables 1 and 2). None of the patients had a history of severe surface disorders, glaucoma or herpetic eye disease.

Grafts

All grafts were preserved in organ culture according to the guidelines of the European Eye Bank Association [32]. Preoperative evaluation of the graft endothelium was performed in hypotonic solution under the phase contrast microscope the day before penetrating keratoplasty (Table 3) [33]. This examination was shown to deliver reproducible results [34].

Penetrating keratoplasty, postoperative treatment and controls

Surgery was performed by three experienced surgeons in retrobulbar anesthesia according to a standardized scheme. Modified Franceschetti trephines with the diameters of 7.5 mm (recipient) and 7.7 mm (donor) were used. Graft fixation was performed with a double running cross-stitch suture with Nylon 10.0 [35]. If necessary, cataract surgery was carried out simultaneously (Table 2). After surgery, gentamycin ointment was administered at least until the graft was covered with a complete epithelial

Table 1. Topical FK506 after normal-risk keratoplasty, indications for surgery.

	Group I (FK506)	Group II (steroid)
Bullous keratopathy after cataract surgery	4	7
Fuchs' endothelial dystrophy	6	10
Avascular, nonherpetic scars	4	2
Keratoconus	6	1

Table 2. Topical FK506 after normal-risk keratoplasty, patient data.

	Group I (FK506)	Group II (steroid)
Age (years)	58 ± 20	70 ± 15
Gender (male/female)	14/6	7/13
Tripel procedures	3/20	4/20
Previous i.o. surgery	5/20	9/20
Follow-up (months)	10.3 ± 4.5	11.0 ± 2.1

Table 3. Topical FK506 after normal-risk keratoplasty, donor/graft data.

	Group I (FK506)	Group II (steroid)
Age (years)	72 ± 17	69 ± 12
Gender (male/female)	11/9	12/8
Postmortem time (h)	30 ± 18	34 ± 20
Organ culture period (days)	17 ± 10	20 ± 5
Preoperative endothelial cell density (cells/mm^2)	2550 ± 200	2282 ± 177
Follow-up (months)	10.3 ± 4.5	11.0 ± 2.1

layer. All patients received 1 mg/kg bodyweight/day of systemic fluocortolon tapered within 3 weeks postoperatively. Acetazolamide was administered in a daily dose of 500 mg for 5 days postoperatively. Controls of the graft at the slit-lamp were scheduled 7 weeks, 4, 12 and 18 months postoperatively and thereafter annually.

Immune reactions

Endothelial immune reactions were diagnosed via endothelial precipitates and stromal edema, stromal immune reactions via subepithelial infiltrates [36]. The patients then received corticosteroid eye drops (prednisolone-21-acetate 1%) every hour until elimination of all precipitates. Furthermore, a subconjunctival injection with betamethasone-21-acetate was performed. Topical corticosteroids were tapered individually. In severe cases, systemic corticosteroids at a daily oral dose of 1 mg fluocortolone/kg bodyweight were administered additionally and tapered within 3 weeks.

Detection of FK506 in the blood

In all patients of the FK506 group, FK506 blood levels were measured 7 weeks postoperatively. This was carried out using the Microparticle Enzyme Immunoassay (MEIA)®. This test determines FK506 and some of its metabolites in whole blood samples. The lower detection limit is 1.5 ng/ml.

Statistical analysis

All statistical evaluation was performed using SPSS Windows NT 4.0 (Microsoft Corp., Redmond, WA, USA). Clear graft survival, ratio of grafts without immune reactions and rejection-free graft survival were calculated according to Kaplan and Meier [37]. Kaplan–Meier curves were compared via log-rank test.

Results

One year postoperatively all patients of the FK506 group without premature withdrawal of the drug were free from immune reactions, in contrast to 84% in the steroid group (Fig. 1a and b; Kaplan–Meier values; $P = 0.9$, log-rank test). None of the patients developed irreversible graft failure so far.

Local side effects were observed in eight patients of the FK506 group (Table 4). They concerned the surface of cornea and conjunctiva. In detail, superficial punctate keratitis, injection of the conjunctiva, burning, an erosion persisting over 10 days and the development of a slight superficial opacification were recorded. All eight patients asked for premature withdrawal of the drug which was performed 3.0 ± 0.5 months postoperatively. Under topical steroids surface disorders improved in all eight patients. After withdrawal of FK506, one of these eight patients developed a reversible endothelial immune reaction.

In the steroid group local side effects were observed in nine patients (Table 4). Here, superficial punctate keratitis, injection of the conjunctiva and the development of a slight superficial opacification were recorded.

Systemic side effects were not observed. In none of the 20 subjects of the FK506 group, FK506 could be detected in whole blood samples.

Discussion

For many years topical steroids have remained the only effective topical immunosuppressive after penetrating keratoplasty [6]. On the one hand, the duration of steroid application depends on the risk of rejection in the

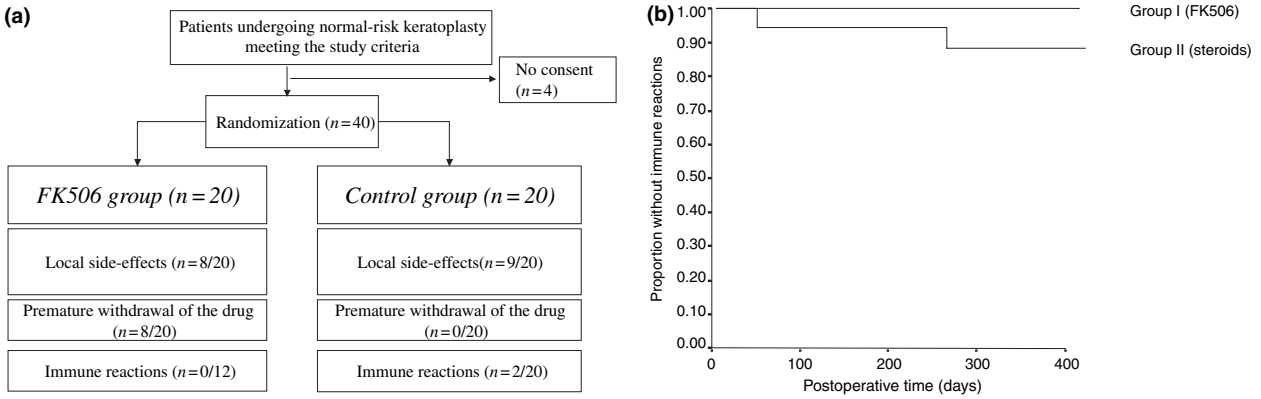

Figure 1 (a) Topical FK506 after normal-risk keratoplasty, consort scheme. (b) Topical FK506 after normal-risk keratoplasty, efficacy (Kaplan–Meier curves, $P = 0.9$, log rank test).

Table 4. Topical FK506 after normal-risk keratoplasty, local side effects.

	Group I (FK506)	Group II (steroid)
Superficial punctate keratitis	8/20	8/20
Injection of the conjunctiva	6/20	2/20
Burning sensation	6/20	0/20
Superficial opacification	2/20	1/20
Erosion	1/20	0/20
No side effects	12/20	11/20

individual patient. On the other, local side effects like steroid response glaucoma, cataract, surface disorders or infections often limit this period. Therefore, in the long run, topical steroids can only be administered, if at all, in a low dose of 1–2 drops per day [7]. Systemic immunosuppression is effective, but may be associated with severe systemic side effects [11–15]. There is a need, therefore, for alternative topical immunosuppressives.

After a single instillation of one FK506 0.1% eye-drop, maximum FK506 levels above 400 ng/ml can be measured in the cornea of male rabbits [38]. Twenty-four hours thereafter, FK506 levels still exceed 100 ng/ml [38]. This is more than 10-fold above effective blood levels after systemic administration in clinical organ transplantation [25,26,39]. In most animal keratoplasty models a concentration of 0.03% was found to be effective without side effects [28–30]. In this pilot study, a concentration of 0.06%, i.e. double as high as in the animal experiments was chosen. With this prophylaxis, none of the grafts in the FK506 group developed immune reactions so far. Thus, FK506 seems to be at least as effective as topical steroids. Possibly, the patients under topical FK506 did not develop immune reactions because the drug might be more effective than topical steroids and might induce more mid-term immune tolerance. We should keep in mind, however, that the study groups were very small and follow-up was limited. Larger (multicenter) studies, therefore, should be performed.

Complications of topical FK506 are disorders of the corneal and conjunctival surface [31]. These were observed in eight of 20 patients. As these patients asked for premature withdrawal of the drug, FK506 application was stopped. These side effects were most probably caused by the drug itself, i.e. FK506. The pH of the solution was neutral and all further ingredients seem unproblematic. Most of these ingredients are used in many eye drop preparations. In a next step, therefore, we intend to test topical FK506 in a concentration of 0.03%. However, in the control group nine of 20 patients showed similar side effects.

In summary, efficacy of topical FK506 was at least as high as of topical steroids in this pilot study. FK506, therefore, might turn out to become an effective immunoprophylaxis in normal-risk keratoplasty patients. Local discomfort should be further reduced.

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