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角膜 移植手術 후 移植 拒否反應 抑制에 대한 cyclosporine A의 有用性

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The effectiveness of topical cyclosporine A to graft rejection in corneal transplantations.

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Abstract

角膜 移植手術 후 移植 拒否反應 抑制에 대한 cyclosporine A의 有用性

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목적: 角膜移植術을 받은 환자에서 국소 cyclosporin A의 점안이 移植 拒否反應에 갖는 有用性을 알아보고자 하였다.

대상과 방법: 2005년 3월부터 2007년 3월까지 角膜移植術을 받은 환자 39명 39안을 3개의 군으로 나누어 cyclosporin A를 투여방법을 달리하면서 移植 拒否反應의 발생을 前向的으로 分析하였다. 국소 steroid와 cyclosporin A를 수술 직후부터 竝用投與한 군이 18명 18안, 수술 후 6개월부터 竝用投與한 군이 10명 10안, 국소 steroid만 점안한 군이 11명 11안 이었다.

결과: 角膜移植術을 받은 39안에서 국소 steroid와 cyclosporin A 0.05%를 竝用 점안한 군이(Group A, Group B) 국소 steroid만을 점안한 군(Group C)과 비교하였을 때 角膜移植 拒否反應이 낮게 발생하였다(P=0.033, 0.042). 그러나 Cyclosporin A 0.05%의 투약시기에 따른 비교에서는 移植 拒否反應에 차이가 없었다(P=0.172). 移植 拒否反應의 발생 시기도 국소 steroid만 점안한 군에서 늦게 발생하는 것으로 나타났지만 이에 대한 통계학적 의미는 없었다(P=0.149).

결론: 角膜移植 拒否反應을 억제하기위해 1% prednisolone acetate와 cyclosporin A를 병용 점안하는 것이 효과적이라 할 수 있을것이다.

Key Words : 角膜移植 拒否反應, 국소 cyclosporin A

Introduction

Keratoplasty is one of the most common organ transplantation procedures, whose success rate is higher in short-term graft success procedures than the other transplantations. But according to recent studies, graft survival rate is lower in long-term graft success than in that of short-term, and it was reported that the success rate of keratoplasty in developing Asian countries was less than 50% in the past decade.[1-3] Immunologic graft rejection is one of the main causes of penetrating keratoplasty(PK) failures, which accounts for approximately 50% of the failures.[4-7] Evidence suggests that transplant rejections caused by the major histocompatibility complex of the keratocyte, but it has still not been clarified as to which region has the strongest antigenicity and how the transplants rejected.

Topical steroid has been traditionally used to prevent transplant rejection, which is known to be effective against even post-transplant rejection. However, the problem is that topical steroid application causes various side effects, such as the delay of wound healing, the increase of intraocular pressure, the onset of cataract and complications on the ocular surface and otherwise.[8-13]Also, steroid use affects collagen formation and thus delays wound healing. In addition, it inhibits immunologic function and increases the possibility of secondary infection. In order to prevent the transplanted cornea from being rejected, long-term steroid application needs to be substituted or reduced by an other effective treatment.

Cyclosporin A is a cyclic polypeptide produced as a metabolite by the mold fungus Tolypodadium inflatum Gams. It is an immunosuppressive drug that inhibits the activity of T-cell transcription factor of activated T cell, interfering with the induction of cytokines and gene expression required for the immune response.[14] Cyclosporin A is widely used systemically in other transplantations.[15,16] But, systemic administration is associated with unavoidable side effects including lethargy, nephrotoxicity, hepatotoxicity and hypertension. Thus in recent years the effectiveness of topical cyclosporin for graft rejection has been an interesting topic of research. A survey of members of "The Cornea Society" suggested that 48% of respondents used

cyclosporin A as prophylaxis for high risk graft group and 7-13% respondents used it treatment for definite graft rejection.[17] Zhao and Jin(1995) presented a series of patients treated with topical cyclosporin A as monotherapy for graft rejection. It appeared to have a beneficial effect in reversing graft rejection in 15 out of 16 patient[18], conversely Price and Price(2006) reported that topcial 0.05% cyclosporin was not as effective as the use of topical prednisolon acetate for prevention of graft rejection.[19] The efficacy of topical cyclosporin A on keratoplasty is still debatable.

This prospective study was evaluated to know the efficacy of topical cyclosporion A on corneal transplant rejection.

Patients and Methods

This study was a single center prospective study with the 39 patients (39 eyes) who underwent keratoplasty in Chosun University Hospital from March 2005 until March 2007. Chosun university institutional review board approvals for the study were obtained, and all patients provided informed signed consent. The study was conducted in accord with good clinical practices and with the Declaration of Helsinki, 1996. The follow-up for the subject was performed for at least 1 year, and the results were prospectively analyzed. Full-thickness corneal graft was performed on 32 patients (32 eyes) and lamellar cornea patch graft was on 7 patients (7 eyes). The mean age of the patients was 57.6 years. The progress was evaluated for at least 12 months, at most 18 months. Bullous keratopathy, corneal opacity by the corneal ulcer and corneal perforation by trauma are considered as the principal causes of corneal transplantation. Most of subjects were classified as a high-risk for graft failure (Table 3). All patients in the study enrolled to three regimens at random after surgery without distinction of risk for graft failure. Table 2 shows the demographics of subjects assigned into 3 groups. Keratoplasty was performed by the one operator. All the corneas for graft were donated within 6 hours after death. For enucleations, after cleaning the eye externally with cotton wool soaked with povidone iodine 10% applied on the skin of the eyelids and then irrigating the conjunctival sac with saline. A 360° peritomy was performed. The 4 quadrants were bluntly dissected between the rectus muscles to separate the tenon capsule from the globe, and all the muscles were individually isolated with a muscle hook. The muscles were then cut from the globe. Dissection was continued posteriorly around the globe and the optic nerve was transected with the use of curved scissors and the globe enucleated. At the end of the procedure, the orbit was packed with moist cotton and the lid was closed with an interrupted 7-0 Black silk suture. The eye were kept in a sterilized wet bottle containing sterile cotton soaked in sterile Balanced Salt Solution Plus (Alcon Laboratories) and transferred to the eye bank of chosun university hospital. The

corneas, judged normal on hemato-immunologic examination, were putted into Optisol (Chiron Ophthalmics, Irvine, California), and were transplanted within 24 hours. Tissue matching for HLA was not performed. All the case of corneal graft was performed under retrobulbar anesthesia and all the patients was injected with 15% mannitol(400mL) for 30 minutes before operation. The donor cornea was trephined from the endothelial surface and trephine size was 0.25-0.50 mm larger than the recipient cornea beds. After a scleral fixation ring was sutured with for interrupted 7-0 Vicryl, thephination of donor and recipient cornea was performed. Furthermore interrupted or continued suture was performed with 10-0 nylon. When the operation was finished, steroid (dexamethasone) and antibiotics (ceftazole) were injected subconjunctivally. To 18 subjects (18 eyes) out of 39, steroid was topically administered concurrently with 0.05% cyclosporin A immediately after the operation (Group A). To 10 subjects (10 eyes), steroid was topically administered concurrently with 0.05% cyclosporin A 6 months after the operation (Group B). And to the other 11 subjects (11 eyes), only steroid was topically instilled (Group C). For the eye-dropping steroid, 1% prednisolone acetate (Pred-Forte®, Allergan, USA) was used, and for the topical cyclosporin A (Restasis®, Allergan, USA) was used (Table 1). After undergoing the operation, all the subjects instilled gatifloxacin 0.5% (Gatiflo®, Handok, Korea) and 1% prednisolone acetate in their eyes 4 times a day. In group A subjects were instilled cyclosporin A four times a day in addition. Predisolone acetate was used for at most 3 months according to postoperative conditions, and was tapered over 12 months. Participants' progress was evaluated every month after surgery. The clinical evaluation at each visit included visual acuity with Snellen chart, slit lamp examination.

Cases where the cornea was edema or keratic precipitates, subepithelial infiltrates and rejection line was observed, were diagnosed as the graft rejection. And when an edema developed in graft cornea and it is opacified and the vision is not returned, the time of the transplant were used to define transplant failure. Diagnosis was entrusted to one specialist, and was based on the results of visual acuity test and slit-lamp microscopy. Failures due to other cause such as glaucoma or infection were excluded

from the analysis.

Statistical analyses were performed using the SPSS statistical software (Version 14.0, SPSS Science, USA). The primary outcome measures were the incidence of immunologic graft rejection episode. The results according the the different treatment regimen were analyzed by use of Kaplan-Meier survival analysis. The cases where the P-value is less than 0.05 ($p<0.05$) were defined as statistical significance. Descriptive statistics are reported as mean values and standard deviation.

Result

In the 18 subjects (18 eyes; Group A) who instilled topical steroid concurrently with cyclosporin A immediately after the operation, transplant rejection occurred in 2 eyes (11%). In the 10 subjects (10 eyes; Group B) who instilled topical steroid concurrently with cyclosporin A 6 months after the operation, the rejection occurred in 1 eye (10%). In the case of the 11 subjects (11 eyes; Group C) who instilled only topical steroid, the rejection was observed in 5 eyes (45%) (Fig.1; Table 2). Also on statistical analysis, there was a significant difference in rejection between the two groups to which cyclosporin A was administered and the other one (p=0.033, 0.042). However, there was no difference between the two groups to which cyclosporin A was administered at different points in time (p=0.172).

In the group to which just steroid was administered, the rejection occurred after 6 months on the average. In the groups to which topical steroid and cyclosporin A were concurrently administered immediately after the operation, the rejection occurred after 3 months averagely. However, there was no statistical significance (p=0.149).

Discussion

Cyclosporin A is a strong immunosuppressant, and mostly acts on T-cells. Additionally it inhibits the formation of IL-2 and INF-ɣ and the activation of cytotoxic T-cells, and prevents antigen presentation.[20-22] Therefore, cyclosporin A is widely used for patients who underwent renal transplantation, heart transplantation or bone marrow transplantation. In addition to them, it is used to treat autoimmune diseases such as uveitis and rheumatoid arthritis.[23,24] Specially it suppress the afferent limb of the immune response, the role of cyclosporin A has primarily been as prophylaxis against graft rejection. For this reason topical cyclosporin A does not have beneficial effect in treatment of graft rejection when intensive steroids are already being used[25]

Topical cyclosporin A (Restasis®, Allergan, USA) used in this study has been made commercially available after Food and Drug Administration (USA) approval for treatment of dry eye. It is hydrophobic emulsion containing 0.05% cyclosporin A in solution. It gets prolonged contact with the lipophilic surface of the cornea, and has high absorption. So the topcial cyclosporin A readily penetrates the epithelium and accumulates in the stroma. However, cyclosporin A is hardly penetrate into the anterior chamber because it does not easily percolate water-soluble environment. According to Allergan's clinical trial (PK-98-074), the average concentration in corneas was 1550 ng/ml when 0.05% cyclosporin A was instilled in the eyes of rabbits 2 times a day, but that in anterior chambers was remarkably low (1.4 ng/ml). So topcial cyclosporin A penetrated into ocular tissues at concentration adequate for local immunomodulation, whereas penetration into intraocular tissues was much less and absorption into the blood was minimal. The restasis dry eye studies showed that all the patients had less than or equal to 0.16 ng/mL in the blood level. By comparison, the blood level of patients with systemic cyclosporin A medication is in the ragne of 75-360 ng/mL. The benefit of topical cyclosporin A was conferred without systemic side effect.[26,27]

There is controversy as to how long cyclosporin A should be used, but ordinarily

it is known that to be completely immunosuppression effect may take 3 to 6 months. It is known that it is advisable to use cyclosporin A, which inhibits initial immune response, for 6 to 12 months. Also in this study, cyclosporin A was administered 4 times a day for at least 6 months in order that immunity might be inhibited sufficiently. And it appeared that drug concentration enough to inhibit transplant rejection was maintained by what cyclosporin A is administered 4 times a day.

Topcial cyclosporin A use after keratoplasty may have other advantages. It is thought to be effects on subconjunctiva and lacrimal gland inflammation by inhibiting lymphocyte infiltration in the lacrimal and conjuntiva. It also has effect on increase in tear production and conjunctival goblet cell density.[28] It has been shown to have antifungal and antiviral activities against several viruses in vitro compared with corticosteroid. Topical cyclosporin A modulates the local immune response by supressing antigen-activated T lymphocyte while preserving the immune system's antimicrobial action.[29-31]

Recently some study showed the combination treatment of commercially available topical cyclosporin 0.05% and steroid or the only topcal cyclospin A treatmet are not beneficial on graft rejection in low and high risk corneal graft patient.[19,32]But in the current study, we find that combination treatment of 1% prednisolone acetate and 0.05% cyclosporin A is effective for the inhibition of graft rejecitions significantly in statistics.

However, there were several problems with this study. First, subjects were of small number. Second, the factors that might affect statistical results, such as causal diseases and drug compliance, were not completely excluded. Lastly there was no group to instill only cyclosporin A, and as a result the independent administration of cyclosporin A was not compared with the combination of topical steroid and cyclosporin A. An additional study needs to be performed with a large number of subjects with different concentration of topcial cyclosporin A in the near future.

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Table 1. Treatment Regimen

Regimens	A	B	C
Start of topical CsA	Immediately	6 months	No
Oral predisolone discontinued	3 months	3 months	3 months
Topical predisolone tapering start	3 months	3 months	3 months
Topical Predisolone discontinued	12 months	12 months	12 months

PK = penetrating keratoplasty

CsA=Cyclosporine A

Table 2. Patient's distribution and failure rate

	Group A	Group B	Group C
Subject (n)	18	10	11
Male: Female (n)	11: 7	8: 2	11: 0
Age (yrs) mean	54.03	48.11	44.27
Rejection episode n (%)	2 (11)	1 (10)	5 (45)

Table3. Preoperative diagnosis

Preoperation Diagnosis	Group A	Group B	Group C
Keratoconus, n (%)	1 (5)	0 (0)	1 (9)
Corneal dystrophy, n (%)	0 (0)	1 (10)	1 (9)
Bullous keratopathy, n (%)	2 (11)	3 (30)	5 (45)
Corneal opacity*,n(%)	4 (22)	3 (30)	2 (18)
Corneal perforation, n (%)	3 (16)	3 (30)	1 (9)
Corneal ulcer, n (%)	8 (44)	0	1 (9)

* Corneal opacity of unknown etiology

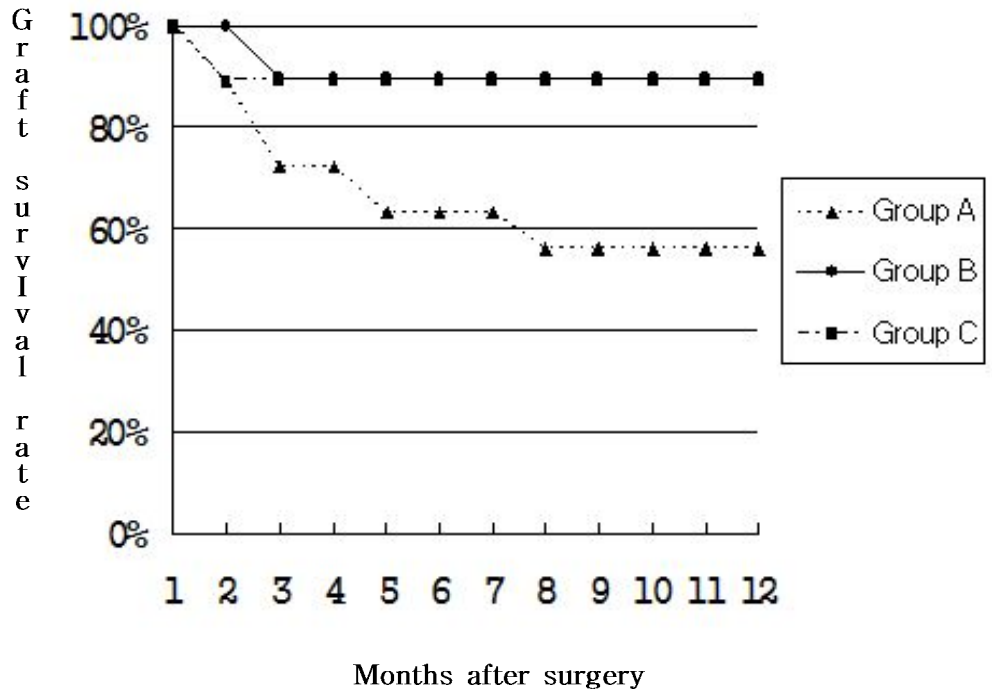

Figure 1. Rejection free survival curves for subjects to regimen A, B and C. (Group A: Topical steroind, Group B : Topical steroid + Cyclosporine A 6months after PK, Group C: : Topical steroid + Cyclosporine A immediately after PK)