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Clinical Efficacy of Topical Homologous Fibronectin in Persistent Corneal Epithelial Disorders

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The clinical efficacy was investigated of topical homologous fibronectin on persistent corneal epithelial defects of various etiologies. Fibronectin was purified from blood bank homologous plasma by gelatin-Sepharose 4B affinity chromatography. Twenty eight eyes of twenty five patients with persistent corneal epithelial defects and sterile corneal ulcers that failed to improve with standard therapy were treated by the instillation of homologous fibronectin eyedrops 5 times a day (500 μg/ml). Complete reepithelialization was achieved in all patients except two eyes due to uncontrolled glaucoma and the taking of steroids. The healing time tended to be different depending on the duration of persistent corneal epithelial defects and the severity of underlying diseases. The mean ± standard deviation duration of epithelial defect was 68.18 ± 77.80 days. Average healing time was 42.07 ± 17.47 days. Ocular symptoms were relieved significantly and no side effects were observed. Over an average follow-up period of about 8 months, two cases of recurrences were noted. These results show that homologous fibronectin was also effective in patients with persistent corneal epithelial defects and corneal ulcers.

Key words: homologous fibronectin, corneal epithelial defects, corneal ulcers.

INTRODUCTION

The biological significance of fibronectin in wound healing has been studied extensively. This glycoprotein with a molecular weight of 440 K dalton appears at the corneal wound and disappears when epithelial wound healing is completed.[1-4] The facilitative effects of fibronectin on corneal epithelial migration have been reported in rabbit cornea.[5,6] Autologous fibronectin eyedrops were first purified by Nishida *et al*[7] and were proven to promote epithelial healing in humans with persistent corneal defects.

Several authors and we[8-13] have experienced the fact that autologous fibronectin eyedrops were effective in reepithelialization of eyes with persistent corneal epithelial defects. However, production of human autologous fibronectin, whenever needed, is very laborious and lengthy. So, we studied the efficacy of homologous fibronectin in patients with refractory persistent epithelial defects and trophic corneal ulcers.

MATERIALS AND METHODS

Twenty eight eyes of twenty five patients with refractory corneal epithelial defects were followed up at the Department of Ophthalmology,

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Table 1. Patient Data

Case No./ Sex/Age (years)	underlying diseases	D	F	H	PrV	PoV	R
1/f/74	recurrent corneal erosion (postcataract surgery)	46	21	35	0.2	0.5	—
2/m/34	herpetic corneal ulcer	42	21	—*	CF10	—	—
3/m/54	herpetic keratitis secondary glaucoma	245	21	63	0.05	0.1	—
4/m/70	herpetic keratitis	360	21	62	0.2	0.5	—
5/m/64	corneal ulcer	35	21	42	HM	CF10	—
6/m/38	corneal ulcer	24	21	25	HM	HM	—
7/m/61	corneal ulcer	120	21	64	HM	0.05	—
8/m/11	chemical keratitis (silver nitrate)	84	21	31	0.3	1.0	—
9/m/44	herpetic corneal ulcer	98	40	62	0.3	1.0	—
10/f/76	chemical keratitis (od)	28	21	34	0.1	0.2	—
11	(os)	21	21	42	0.08	0.2	—
12/m/1	corneal ulcer (after (od)	53	41	13	—	—	—
13	measles infection) (os)	53	77	33	—	—	—
14/m/50	herpetic keratitis	104	63	12	0.06	HM	+
15/m/65	corneal ulcer senile mature cataract	23	42	37	0.01	0.01	—
16/m/29	corneal thermal burn	31	45	75	HM	HM	—
17/m/74	corneal ulcer secondary glaucoma	171	35	—**	LP	LP	—
18/f/31	blepharokerato-conjunctivitis	31	23	48	0.3	0.8	—
19/m/27	chemical corneal burn	101	43	50	0.8	1.0	—
20/m/08	vernal keratoconjunctivitis	9	28	32	0.3	0.8	—
21/m/43	thermal corneal burn	17	42	24	HM	0.2	—
22/m/62	recurrent corneal erosion hyperthyroidism	50	20	24	0.1	0.5	—
23/f/44	corneal ulcer	28	22	41	0.15	0.5	—
24/f/57	corneal ulcer	13	21	35	0.3	0.4	—
25/f/43	corneal ulcer	27	44	58	CF10	0.04	+
26/m/28	chemical corneal burn (od)	28	84	41	CF10	0.04	—
27	(os)	28	84	67	0.1	0.5	—
28/m/72	herpetic geographic ulcer	39	45	21	CF10	CF10	—

D: duration of epithelial defects
F: duration of fibronectin treatment
H: period from beginning of treatment until epithelial healing
PrV: visual acuity before fibronectin treatment
PoV: visual acuity after fibronectin treatment
R: recurrence
*: He had taken steroids at drug store without permission. At last, his cornea perforated
**: failed to heal due to the uncontrolled glaucoma

Dongsan medical center, Keimyung university from June 1988 to Dec. 1989. The persistent epithelial defects were secondary to different underlying conditions; corneal ulcers (8 patients 8 eyes), recurrent herpetic keratitis (6 patients 6 eyes), chemical keratitis (3 patients 4 eyes), corneal burn (3 patients 4 eyes), multiple corneal erosions (3 patients 3 eyes), postmeasles keratitis (1 patient 2 eyes) and vernal keratoconjunctivitis (1 patient 1 eye) (Table 1). In cases of corneal ulcer, no organisms were found on culture and smear.

Patients ranged in age from 1 to 76 years (mean, 46.40 ± 21.61 years). Six patients were female and nineteen patients were male. Their defects had persisted for 9 to 360 days (average 69.70 ± 78.85 days) before fibronectin treatment and had been refractory to conventional medical therapy such as pressure patch, lubricants, topical antibiotics, artificial tears, antiviral agents, topical corticosteroids, and therapeutic soft contact lenses. Toxic antivirals and aminoglycosides were discontinued in a patient with herpes simplex virus keratitis without improvement.

All patients received topical fibronectin, 500 μ g/ml, five times a day. Duration of fibronectin instillation was from 21 days to 77 days (average 36.04 ± 19.78 days). Concurrent therapy consisted of all preexisting therapeutic modalities and medications in similar or decreased dosages. The patients were examined 3 or 4 times a week during administration of fibronectin and then, every week after discontinuation of fibronectin therapy. They were asked to report any new ocular symptoms and signs that developed in the intervals between required study visits. At all visits, they were monitored by slit-lamp biomicroscopy and with serial photographs of fluorescein-stained epithelial defects. Examination included measurements of best corrected visual acuity, extent of eithelial defects, characteristics of the epithelial edges, time of complete healing, and assessment of side effects such as infections or allergic reactions.

Fibronectin was purified by affinity chromatography as previously described by Kim *et al*[9] from homologous blood bank plasma which was screened for syphilis, hepatitis, and AIDS, and was confirmed free of these diseases. Plasma was prepared by centrifugation of citrated blood at 20,000 g. Plasma was filtered at room temperature through unsubstituted agarose, that is Sepharose 4B, to remove particulate matter and agarose-binding proteins. The effluent from this column was allowed to flow directly onto a bed of gelatin-Sepharose 4B. The adsorbed fibronectin was eluted with a freshly prepared solution of 3 M urea in the same buffer and immediately dialyzed against phosphate buffered saline and then lyophilized.[7,8] Fibronectin taken from the above procedures has proven to be pure by SDS-polyacrylamide gel electrophoresis and immunoelectrophoresis. All fibronectin preparations were sterile when tested bacteriologically on blood agar plates, chocolate agar plates, and in thioglycolate broth before use.

RESULTS

A summary of results is shown in Table 1 including underlying diagnosis, duration of defect, duration of fibronectin treatment, healing time, comparision of visual acuity, and recurrences for each of the subjects. Complete or nearly complete healing was achieved in all patients except the following cases. In case 2, his cornea was perforated because he had taken corticosteroids from a local drug store without permission on the 27th day after fibronectin treatment. In case 16, his defect healed completely but with severe vascularization of the cornea. Case 17 had no epithelial healing possibly due to uncontrolled glaucoma.

Mean duration of persistent epithelial defects of 28 eyes was 68.18 ± 77.80 days. The mean healing time from the beginning of fibronectin treatment until healing of the epithelial defect was 42.07 ± 17.47 days. The average duration of fibronectin treatment was 36.04 ± 19.78 days. The correlation coefficient of the duration of defect with the healing time was 0.40358 and statistically significant ($p < 0.1$) (Table 2). Healing rates according to underlying causes were 100% except in eyes with corneal ulcers which had an 87.5% healing rate. In eyes with herpetic keratitis, corneal ulcer and corneal burn, it took a longer time to heal because there seemed to be marked damage to structures beneath the epithelial basement membrane (Table 3). During the average follow-up period of 7.86 ± 4.06 months (2-14 months), two cases of recurrences (case 14, 25) were observed that were due to uncontrolled

Table 2. Statistical data on 28 eyes of 25 patients

Data	Mean ± SD
Age (years)	46.40 ± 21.61
Duration of nonhealing epithelial defect (days)	68.18 ± 77.80
Period until epithelial healing (days)	42.07 ± 17.47*
Duration of fibronectin treatment (days)	36.04 ± 19.78

*$p < 0.1$, correlation coefficient 0.40258

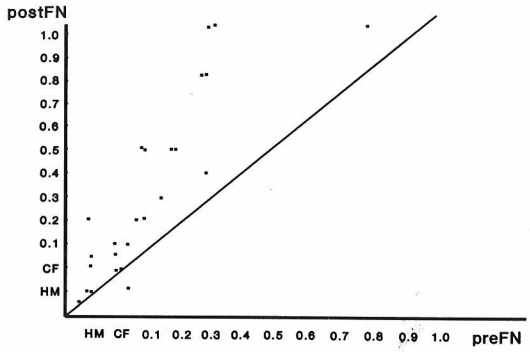

Fig. 1. Comparison of visula acuity before and after fibronectin (FN) treatment.

Table 3. Clinical efficacy of homologous fibronectin eyedrops on the corneal disorders after various causes of corneal diseases

Causes	No. Cases	E (%)	D	H
Herpetic Keratitis	5/6*	100	148	44.0
Corneal Ulcer	7/8	87.5	55.1	43.1
Corneal Burn	4/4	100	26.0	51.8
Chemical Keratitis	4/4	100	58.5	39.3
Recurrent Corneal Erosion	3/3	100	42.3	35.7
Measles	2/2	100	53.0	43.0
Allergy (vernal keratoconjunctivitis)	1/1	100	9.0	32.0

E: efficacy
D: duration of epithelial defects (days)
H: period for epithelial healing (days)
*: one case had steroid without leave

underlying disease. But no side effects of homologous fibronectin were found. Fig. 1 shows the change in visual acuity before and after treatment. In all cases except in two failed eyes, visual acuity improved considerably. Two representative cases are shown in Fig. 2.

DISCUSSION

Nonhealing corneal epithelial defects have multiple etiologies and have been treated in variable therapeutic modalities. Unfortunately, none of the treatment options have proved universally effective. We have previously experienced the fact that the addition of topical autologous fibronectin to persistent corneal epithelial defects was effective in promoting epithelial healing. Based on this positive study, as well as other studies in which fibronectin appeared to promote healing, the present study was initiated.

The results of this preliminary trial suggest that homologous fibronectin may be effective and safe in the treatment of refractory, nonhealing defects of the corneal epithelium, which supports the previous observations of Spiegelman *et al.*[14] The pathobiology of persistent epithelial defects has not yet been clarified. One explanation for the pathobiology is that the basement membrane was damaged during the various diseases, and corneal epithelial cells could not attach to the underlying tissue. It would take more than eight weeks for the basement membrane to be restored. Fibronectin has been reported to be a component of basement membrane, therefore exogenously added fibronectin might act as a film upon which

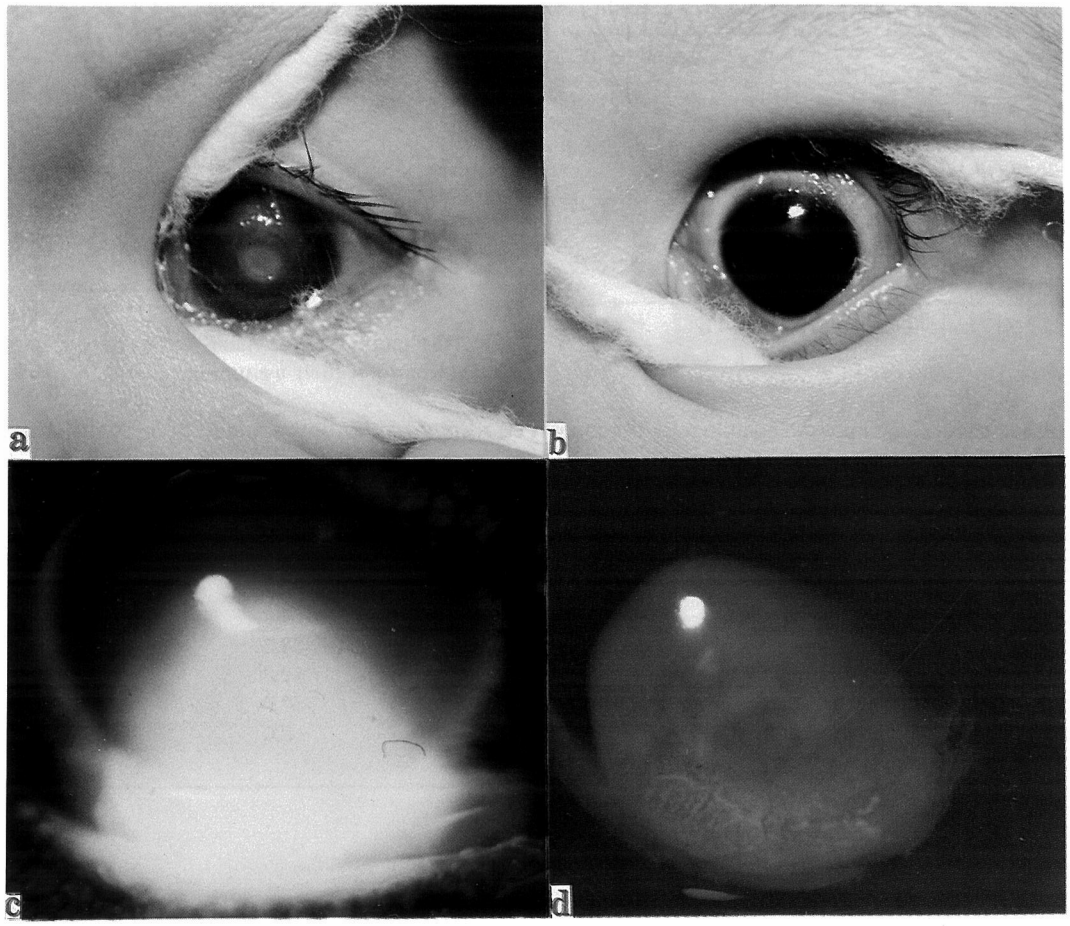

Fig. 2. Photographs of cases 12 and 27. (a) Central epithelial defects after measles infection for 53 days in a 9-month-old infant. Complete healing of epithelium leaving faint corneal scar (b), (c) Conreal and conjunctival alkali burn (35% NaOH) in a 28-year-old man. His visual acuity was 20/200. Complete healing of epithelial defects (d). Visual acuity improved to 20/40.

corneal epithelial cells could migrate. Another possibility is that exogenously given fibronectin would bind to the bared surface of the stromal collagen and also to the fibronectin receptor of the corneal epithelial cells.

Fibronectin was discovered in 1948 by Morrison and co-workers[15] and they termed it "cold insoluble globulin" (CIG). And then, in 1970 Mosesson and Umfleet[16] purified fibroenectin from plasma. And in 1981 Fujikawa *et al*[1] reported the role of fibronectin in healing rabbit corneal wounds. In 1982 rapid preparation of purified autologous fibronectin eyedrops from patient's plasma was presented by Nishida and co-workers.[7] Since then, clinical applications of fibronectin have been tried by Nishida *et al*,[12] Phan *et al*[13] and others. In 1987 Spiegelman *et al*[14] reported the promising effect of homologous fibronectin in patients with persistent corneal epithelial defects.

The functional characteristics of fibronectin have been considered to play an important role in corneal would healing. That is, fibronectin functions as glue between cell-cell or cell-substratum, facilitates cell motility, works as a chemotactic agent, does protein synthesis and mitosis, and stimulates macrophage growth factor activity.[5,17]

Several mechanisms of fibronectin action in corneal wound healing have been demonstrated. Putting all the reports together, those mechanisms were explained by the relation of fibronectin with collagen, fibrinogen/fibrin, plasminogen-plasmin system, actin and ascorbic acid. But they are not yet fully understood.[18]

A drawback of fibronectin eyedrops is the limited stability of biological activity, so pharmacological studies to stabilize the fibronectin molecule are essential, and storage of homologous fibroenectin is an important problem. At present, storage below −4°C after lyophilization of purified fibronectin is thought to be the most stable method.

Topical homologous fibronectin was effective in healing of the corneal epithelial defects in eyes with several underlying diseases refractory to standard therapy in this study. It appears that homologous fibronectin may be a therapeutic alternative for patients with persistent epithelial defect, and although immunologic reactions or other infections were not observed in our study of homologous fibronectin treatment, a more controlled, multi-center, double-blind study is needed.

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