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Topical Fibronectin Treatment in Persistent Corneal Epithelial Defects and Corneal Ulcers

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Topical fibronectin, autologous and homologous, was used to treat nine patients (eleven eyes) with persistent corneal epithelial defects and corneal ulcers that failed to improve with standard therapy. The fibronectin was purified from autologous and homologous plasma by gelatin-Sepharose 4B affinity chromatography and administered topically, 500 μg/ml five times a day, for three weeks. Complete or nearly complete reepithelialization was achieved in all patients regardless of the source of fibronectin, autologous or homologous. But healing times varied. The average healing time was 41.7 ± 14.7 days (35.7 ± 12.4 days for autologous, 50.8 ± 14.4 days for homologous). Ocular symptoms were relieved significantly, and no side effects were observed. Over an average follow-up period of 5.2 months, no recurrences were noted. The results showed that homologous, as well as autologous, fibronectin was effective in patients with persistent corneal epithelial defects and corneal ulcers.

Key words: autologous fibronectin, homologous fibronectin, persistent corneal epithelial defect, corneal ulcer.

INTRODUCTION

Persistent corneal epithelial defects pose a major therapeutic dilemma, and no medications have been universally effective in promoting the healing of these defects. Persistent epithelial defects or corneal ulcers occur in many conditions, most commonly in metaherpetic keratitis, neurotrophic keratitis, anterior segment necrosis, chemical burns, or dry eye syndromes. If left untreated, the defects may be compliated by superinfections or stromal melting and perforation. While most heal with conventional management, such as eyelid taping, lubricants, discontinuation of toxic antibiotics and antiviral medications, soft bandage lenses or tarsorrhaphy, some remain refractory to standard therapy. Fibronectin, formerly known as cold-insoluble globulin, is a plasma and extracellular matrix glycoprotein. Its molecular weight is 440,000, and it has been referred to using various terms, such as multifunctional glycoprotein, large external transformation sensitive protein, surface fibroblast antigen, cell surface protein(CSP), cell adhesive factor(CAF), galactoprotein A(Gap A), zeta protein, opsonic factor, cell spreading factor, and antigelatin factor.[1,2]

Fibronectin is an extracellular structural protein with the unique ability to bind cells and collagen. It plays a major role in the development of the cornea. The universal appearance of fibronectin within eight hours of corneal wounding has promoted major interest in its wound-healing

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properties.[3]

We have therefore estimated that fibronectin may play a critical role in the treatment of persistent nonhealing epithelial defects with damaged stroma and abnormal basement membrane components synthesis.

We and several authors have experienced that autologous fibronectin eyedrops are effective in reepithelialization of eyes with persistnet epithelial defects. So, we report here the results of a trial of homologous, as well as autologous, topical fibronectin in patients with refractory persistent epithelial defects and trophic stromal ulcers of the cornea.

SUBJECTS AND METHODS

Eleven eyes of nine patients with refractory corneal epithelial defects were followed up at the Department of Ophthalmology, Dongsan Medical Center, Keimyung University, from June, 1988 to Aril, 1989. The persistent epithelial defects were secondary to different underlying conditions: keratitis sicca with rheumatoid arthritis (1 patient, 2 eyes), recurrent herpetic keratitis (2 patients, 2 eyes), metaherpetic keratitis (1 patient, 1 eye), disciform keratitis with epithelial defect (1 patient, 1 eye), postoperative corneal erosion (2 patients, 3 eyes), and corneal ulcer(2 patients, 2 eyes)(Table 1). Postoperative corneal erosions were cases after extracapsular cataract extraction with posterior chamber lens implantation and levator muscle resection, respectively, and in the case of corneal ulcers, no organisms were found on culture and smear.

The patients ranged in age from 32 to 74 years-(mean, 54.2 years), and six patients were male and three were female. Their defects had persisted for five weeks to 52 weeks (mean, 16 weeks) before fibronectin treatment and had been refractory to conventional medical therapy, such as pressure patch, lubricants, topical antibiotics, tears naturale, antiviral agents, topical corticosteroids, and therapeutic soft contact lens. Antivirals and aminoglycosides that were thought to be toxic were discontinued.

All patients received topical fibronectin, 500 μ g/ml, five times a day for 21 days. Four of those patients (six eyes) were treated with autologous fibronectin, and five patients (five eyes) with homologous fibronectin. Concurrent therapy consisted of all preexisting therapeutic modalities and medications in similar or decreased dosages. The patients were examined three or four 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 the required study visits. At all vistis, 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 epithelial defects, characteristics of the epithelial edges, time of complete healing, and assessment of side effects, such as infections or allergic reactions.

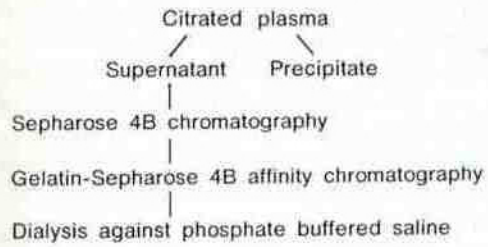
Fibronectin purification

Fibronectin was purified from each patient's autologous plasma and homologous pooled plasma of blood bank which was screened for syphilis, hepatitis and AIDS, and was confirmed free of these diseases.

Fibronectin was purified by affinity chromatography. Then, 0.38% citrated blood was obtained and plasma was prepared by centrifugation at 20,000 g. The 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. All gels were equilibrated in 0.05 M tris-HCl PH 7.5 containing 0.05 M e-amino caproic acid, and the column was washed with 1 M sodium chloride in buffer followed by equilibration buffer to remove nonbinding proteins and minor contaminants. 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 for longer storage (Table 1)[4].

Fibronectin taken from the above procedures has proven to be pure by SDS-polyacrylamide gel elctrophoresis and immunoelectrophoresis. All fibronectin preparations were sterile when tested bacteriologically on blood agar plates, chocolate agar plates, and in thioglycolate broth before use.

Table 1. Schematic drawing of schedule purifying plasma fibronectin



RESULTS

A summary of the results is shown in Table 2, including underlying diagnosis, duration of defect, source of fibronectin, healing time, comparision of visual acuity, and follow-up periods for each of the subjects. In the case of metaherpetic keratitis, the patient had taken corticosteriods at a local drug store without permission on the 27th day after fibronectin treatment. So, the size of the epithelial defect had been decreasing, but then his cornea began to thin and perforated 13 days later. Except for this case, complete or nearly complete healing was achieved in all patients regardless of the source of fibronectin.

As seen in Table 3, the mean duration of persistent epithelial defects of 11 eyes was 109.2 ± 108.1 days. The mean healing time from the beginning of fibronectin treatment until healing of the epithelial defect was 41.7 ± 14.7 days: 35.7 ± 12.4 days for autologous, and 50.8 ± 14.4 days for homologous fibronectin respectively. There may be a tendency to take a longer time for the homologous fibronectin treatment than the autologous fibronectin treatment. However, the difference between both healing times was statistically insignificant ($P > 0.05$). The longer healing time in the homologous fibronectin treatment was probably due to the longer duration of the defect. Because of the small number of cases studied, however, we can't actually conclude that the differences are insignificant, but we can find

Table 2. Effects of fibronectin on corneal epithelial wound healing

Case Age / Sex	Underlying Diagnosis	Duration of Defect(wks)	Fibronectin	Defect Healed (day)
49 / F	Keratitis sicca	27	Autologous	39(OD),25(OS)
58 / M	Recurrent Herpetic Keratitis	14	Autologous	24
53 / F	Corneal Ulcer	5	Autologous	8week
32 / M	Postoperative Erosion	7	Autologous	28(OD), 42(OS)
74 / F	Postoperative Erosion	7	Homologous	35
34 / M	Metaherpetic Keratitis	6	Homologous	—
54 / M	Disciform Keratitis	35	Homologous	9 week
70 / M	Recurrent Herpetic Keratitis	52	Homologous	9 week
64 / M	Corneal Ulcer	5	Homologous	6 week

Case	Visual Acuity		Follow-up Period
Age / Sex	Pre-fibronectin	Post-fibronectin	(months)
49 / F	20 / 50(OD), 20 / 40(OS)	20 / 20(OU)	9(OD), 6(OS)
58 / M	20 / 400	20 / 30	8
53 / F	HM	20 / 200	5
32 / M	20 / 80(OD), 20 / 100(OS)	20 / 20(OD), 20 / 25(OS)	5(OU)
74 / F	20 / 100	20 / 40	4
34 / M	CF at 10cm	—	—
54 / M	20 / 400	20 / 100	4
70 / M	20 / 100	20 / 40	3
64 / M	HM	CF	3

Table. 3. Comparison of autologous and homologous fibronectin, mean ± SD, day

	Defect Healed	Duration of Defect
Autologous	35.7 ± 12.4	101.5 ± 71.1
Homologous	50.8 ± 14.4	173.3 ± 159.3
Total	41.7 ± 14.7	109.2 ± 108.1

that homologous fibronectin, as well as autologous fibronectin, appeared to promote the healing of the corneal epithelium. During the average follow-up period of 5.2 months(3-9 months), no recurrences or side effects were found. Figure 1 shows the change in visual acuity before and after treatment. In all cases except one perforated eye, visual acuity improved considerably. Four representative cases are described through photographs and legends in Fig. 2.

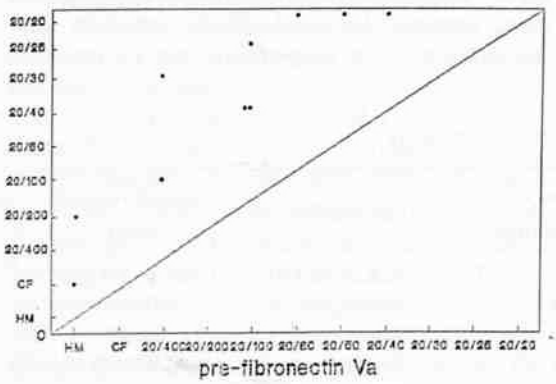


Fig. 1. Comparison of visual acuity.

DISCUSSION

Nonhealing corneal epithelial defects have multiple etiologies and have been treated in a variety of ways. Unfortunately, none of the treatment options have proved universally effective. We have previously experienced that the addition of topical autologous fibronectin to persistent epithelial defects was effective in promoting epithelial healing.[4] Based on this positive study, as well as other studies[5-8] in which fibronectin appeared to promote healing, the present study was initiated.

Results of this preliminary trial suggest that homologous, as well as autologous fibronectin may be effective in the treatment of refractory, nonhealing defects of the corneal epithelium, which supports the previous observations of Nishida et al.[5,6] and Spiegelman et al.[9]

Fibronectin was discovered in 1948 by Morrison and coworkers[10] and they termed it "cold insoluble globulin(CIG)." Then, in 1970, Mosesson and Umfleet[11] purified fibronectin from plasma. In 1981, Fujikawa et al.[12] reported the role of fibronectin in healing rabbit corneal wounds. In 1982, a rapid preparation method of purified autologous fibronectin eyedrops from patient's plasma was presented by Nishida and coworkers.[13] Since then, clinical applications of fibronectin have been tried by Nishida,[7] Phan[8] and others. In 1987, Spiegelman et al.[9] reported the promising effect of homologous fibronectin in patients with persistent corneal epithelial defects.

The functional characteristics of fibronectin are considered to play an important role in corneal wound healing. That is, fibronectin functions as the 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.[14,15]

Several mechanisms of fibronectin action in corneal wound healing have been demonstrated. First, fibronectin was adsorbed to collagen and may constitute the normal substratum for cell adhesion and migration. Second, the addition of fibronectin promotes the adhesion of fibroblasts to fibrinogen or fibrin, while exogenous plasma fibronectin initiates synthesis of fibrin by corneal endothelial cells grown in tissue culture without serum. These showed the direct interaction of fibronectin and fibrinogen/fibrin, which may be important in corneal wound healing. Third, exogenous fibronectin replenishes degraded native fibrin and promotes epithelial adhesion and wound healing. Fourth, fibronectin forces the actin to reorganize intracellular actin filaments to initiate epithelial migration. Last, ascorbic acid may be a necessary cofactor for the final steps in the synthesis and excretion of fibronectin, but these processes are not yet fully understood.[3]

Endogenous sources of fibronectin in corneal wound healing have been the subjects of attention for numerous researchers. As reported, up until now, tears, aqueous humor, limbal blood vessels, retrocorneal fibrous membrane, and the

cornea itself were found to be sources of fibronectin. The cornea itself has been suggested as the major source of fibronectin in corneal wound healing. However, it is uncertain which type of corneal cells are mainly responsible for production of fibronectin. Probably those cells adjacent to the injury might be the source.[16,18]

Topical autologous and homologous fibronectin were effective in healing of the corneal epithelial defects in eyes with several underlying diseases refractory to standard therapy in this study. While these data must be confirmed in a larger series, it appears that fibronectin may be a therapeutic alternative for patients with persistent epithelial defects, and a more controlled double-blind study is needed. In our study of homologous fibronectin treatment, immunologic reactions and other infections were not observed. Production of human autologous fibronectin is laborious and lengthy. So we hope that homologous fibronectin will be produced on a commercial scale in the near future.

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LEGENDS FOR FIG. 2

Case 1. A 58-year-old man complaining of photophobia and visual disturbance (OD) for 14 weeks was found to have corneal epithelial defects secondary to recurrent herpetic keratitis. His visual acuity was 20 / 400, but 24 days after autologous fibronectin treatment, the epithelial defects completely healed and visual acuity improved to 20 / 30.

Case 2. A 53-year-old woman had a nonhealing epithelial defect refractory to treatment for a culture-negative presumed bacterial ulcer in her right eye for five weeks. Her visual acuity was hand motion. Eight weeks after autologous fibronectin treatment, the epithelial defect healed completely and visual acuity improved to 20 / 200.

Case 3. A 54-year-old man with a history of herpes simplex virus keratitis (OD) developed corneal stromal edema, epithelial defects, uveitis, and secondary glaucoma which failed to heal for 35 weeks with standard therapy, so he was treated with homologous fibronectin. Concurrent therapy consisted of eyelid taping, lubricants, topical gentamycin, artificial tears, and 1% Bentos. Nine weeks after homologous fibronectin treatment, the epithelial defects healed completely and stromal edema subsided. The best corrected visual acuity improved from 20 / 400 to 20 / 100.

Case 4. A 74-year-old woman with a history of extracapsular cataract extraction with posterior chamber lens implantation had nonhealing epithelial defects (OS) refractory to conventional therapy for seven weeks. Thirty-five days after homologous fibronectin treatment, her best corrected visual acuity improved from 20 / 100 to 20 / 40 and the epithelial defects healed completely.

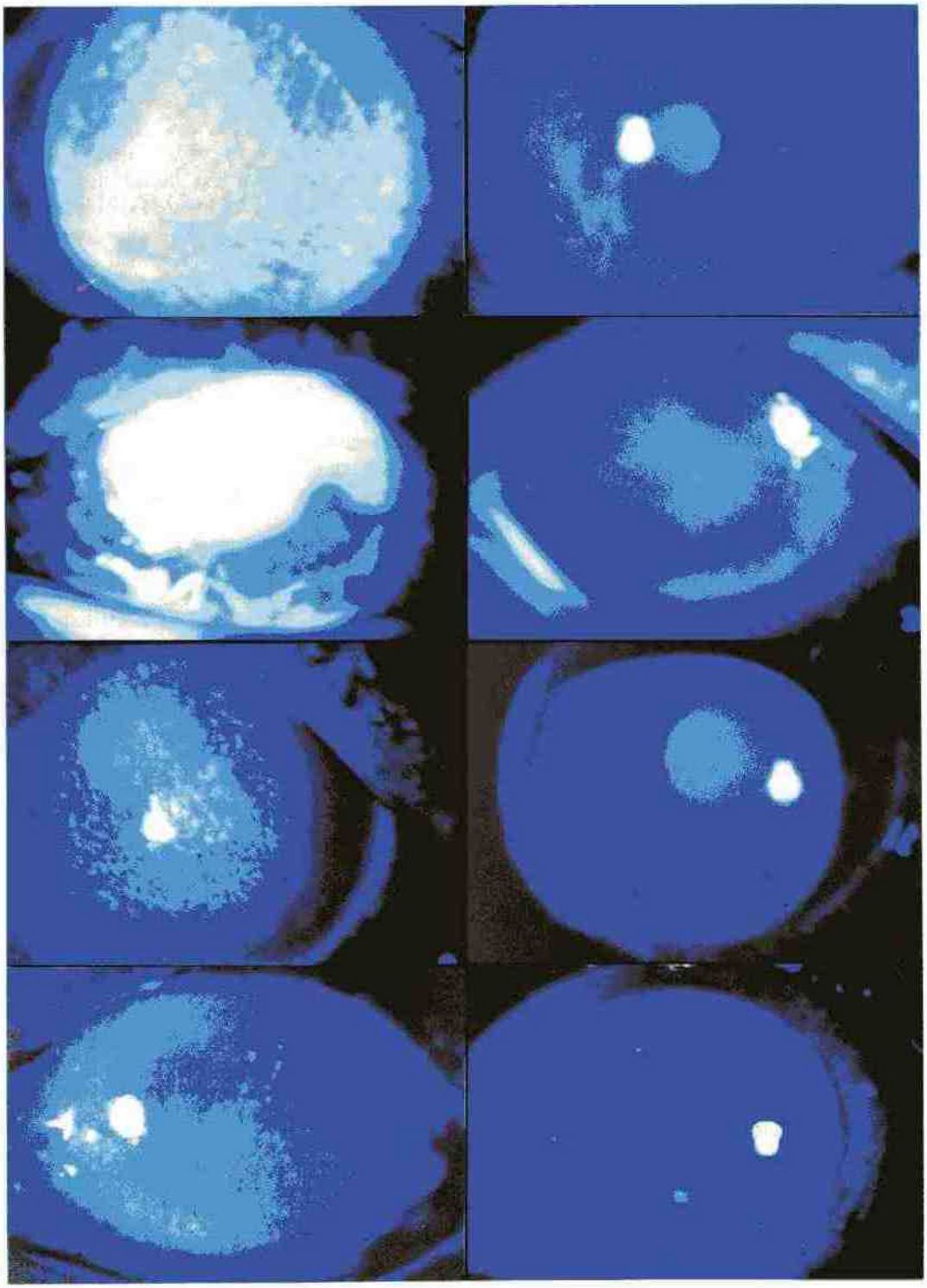

Fig. 2. Photographs of Cases 1 to 4 from top to bottom in order; prefibronectin (left), postfibronectin (right).