TOPICAL FIBRONECTIN THERAPY OF PERSISTENT CORNEAL EPITHELIAL DEFECTS

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INTRODUCTION

PERSISTENT NONHEALING CORNEAL EPITHELIAL DEFECTS (PCED) CAN be defined as a loss of the integrity of the corneal surface, caused by injury or disease, which does not heal within the usual time frame, but persists for weeks or even months. Stromal ulceration may or may not be associated with PCED. Results of basic research in the mechanisms of wound healing and the role of fibronectin (FN) therein as well as of preliminary clinical studies support the premise that topical administration of FN may be efficacious in treating such corneal defects.[1-9]

Underlying disease states that may result in such defects include previous herpes simplex or herpes zoster infection, neurotrophic keratitis after damage to or loss of the fifth cranial nerve function, and mucin-deficient dry eye states occurring after chemical injuries in patients with Stevens-Johnson syndrome or in patients with ocular cicatricial pemphigoid. Nonhealing corneal epithelial defects may also occur after ocular surgery or other physical injuries to the cornea. Cavanagh and associates[10] have provided a good description of the condition.

FN is a protein present in plasma at a concentration of 300 μ/ml. It is a large molecule of 440,000 daltons molecular weight having a number of functional domains, including binding sites for collagen and for cells. FN is an adhesive glycoprotein, which is a normally occurring component of the basement membrane. In addition to its activity as a binding agent that promotes cell-to-cell and cell-to-substrate adhesion, FN also plays a role in

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cell migration, wound healing, and cytoskeletal organization.[11,12] Therefore, it may promote healing by providing the attachment for regenerating epithelial cells and by acting as a stimulus for migration of these cells.[13-17]

A method of purifying FN from plasma and rendering it safe from bloodborne virus infectivity resulting in a preparation suitable for human administration has been developed at the New York Blood Center by Horowitz and Chang.[18] This preparation has been used since 1983 in a number of clinical studies in the United States. It has been shown to be safe and efficacious in several clinical trials.[1-9] It has been shown to promote the attachment of rabbit corneal endothelial cells to plastic culture dishes.[19] The addition of FN to cultures of transformed cells allows the cells to adhere to and move across the surface of the plastic culture plates.[20,21] In vitro studies have shown that endogenously produced FN forms a matrix over the underlying stroma of corneas subjected to superficial keratectomy, thus promoting epithelial cell migration.[22-24] The in vivo induction of corneal lesions has also been shown to be accompanied by FN deposition during the subsequent healing period.[25-28] The presence of endogenous FN on the surface of the injured cornea led to clinical application of exogenous FN in patients with nonhealing epithelial defects, with encouraging results.[6,8] Kono and associates[9] reported on the use of autologous FN in treating a group of 12 patients with keratoconjunctivitisicca (KCS) associated with Sjögren's syndrome; treatment with FN resulted in a reduction in rose bengal and fluorescein staining as well as improvement in the subjective symptoms of dry eye. Nelson and the Chiron KCS-Study group[29] reported on the use of autologous FN in treating a group of 272 patients with KCS, where treatment with FN had no greater therapeutic efficacy in patients with KCS than the vehicle solution or Liquifilm Tears, a commercially available artificial tear solution.

On the basis of data suggesting a role for FN in the healing of ocular surface disorders, a series of studies was initiated, including a multi-center, double-masked placebo-controlled prospective clinical trial to evaluate the safety and efficacy of FN ophthalmic solution in the treatment of patients with nonhealing corneal epithelial defects, to determine whether different etiologies of the defect influence responsiveness to FN treatment, and to evaluate the effect of various concomitant medications on FN effectiveness.

MATERIALS AND METHODS

STUDY MEDICATIONS

The active material was purified human FN prepared at the New York

Blood Center from plasma pools and treated with the organic solvent tri(n-butyl) phosphate and Tween-80 to inactivate lipid-enveloped virus, including hepatitis B, hepatitis C, and human immunodeficiency virus (HIV).[18] It has previously been safely administered either intravenously or topically to more than 100 patients in several studies.[2-5] It was a sterile lyophilized preparation, which when reconstituted, contained 3 mg FN and 3 mg albumin in 3 ml of 0.01 M sodium phosphate buffer, pH 7.4 containing 0.12 M sodium chloride and 0.005 M sucrose. The FN was reconstituted and transferred to an eyedropper bottle just prior to dispensing to the patient, stored at 2° to 8°C (36° to 46°F), and used for a 1-week period only after first use.

The placebo was a sterile, liquid preparation containing albumin prepared by dilution of an FDA-approved albumin. Each vial contained either 6 mg (phase II) or 3 mg (phase III) albumin in 3 ml of 0.01 M sodium phosphate buffer, pH 7.4 containing 0.12 M sodium chloride and 0.005 M sucrose. As with the FN preparation, the albumin was transferred to an eyedropper bottle, stored in the cold, and used for up to 1 week.

Neither preparation contained a preservative. When used in the masked study, each eyedropper bottle was labeled with a code to obscure its identity from both the patient and the treating physician.

PATIENTS

Patients had to be 18 years or older, of either sex and any race, with a reliable history of PCED with or without corneal ulceration (with clinical characteristics of a static epithelial defect) present for more than 2 weeks despite appropriate medical therapy. Children and pregnant or breast-feeding women were excluded. Also excluded were patients experiencing an active ocular infectious process of any sort (eg, live herpes simplex virus infection). Details of the study were explained to eligible patients by the on-site investigator, and the patients were asked to sign informed consent forms.

The patient underwent a pretreatment evaluation consisting of a complete medical history, including details of previous or underlying disease states that may have caused the PCED as well as all medications previously or currently used and a complete ocular examination. The size of the lesion was documented both diagrammatically and photographically after fluorescein staining. Blood was drawn for screening for HIV antibody and for markers of hepatitis B infection, and the patient was later informed confidentially if the results were positive.

STUDY DESIGN

Protocol of Early Phase II (ie, "open-label" trial)

The open-label portion of the study, (early phase II) took place between July 1987 and October 1988, when 25 patients with 27 PCED yielded 26 evaluable corneas. The patients continued their current medical therapy. Each patient received a full ophthalmic examination on day 1; photos with and without fluorescein were taken, and FN (open label) was dispensed, one drop to the affected eye four times a day. Patients were evaluated weekly for up to 4 weeks after healing. Efficacy was defined as 100% healing at any time.

Revised Protocol of Late Phase II (ie, double-masked placebo-controlled)

Between October 1988 and July 1989 a randomized, double-masked, placebo-controlled trial was conducted. Twenty-seven patients were enrolled with 27 PCEDs, 24 of which were evaluable. Patients were treated with either FN or the albumin-containing vehicle, placebo, for 7 to 10 days, and were then evaluated for healing, at which time a patient was given open-label FN if the defect had not healed to an extent of at least 50%. If it had healed to at least 50%, the patient remained in the original treatment. Treatment then continued until 2 weeks after the defect had healed or for 4 weeks, whichever was shorter. The end point for evaluation of FN efficacy was thus whether or not there occurred at least 50% healing of the defect in 7 to 10 days or 100% healing on the masked preparation.

Revised Protocol of Early Phase III (ie, double-blind placebo-controlled after initiation of standardized concomitant therapy)

This clinical study of the administration of FN eye drops to patients suffering from nonhealing corneal epithelial defects was carried out at six active centers throughout the United States (the FN study group [Table I]). A "washout" period of 1 week preceded the entry into the trial. The patient was put on standard medications containing a few preservatives as possible, and excluding BAK, which is a preservative known to interfere with wound healing. If substantial healing occurred during the washout period, the patient was excluded.

The concomitant ocular medications being administered, which were standardized to lack preservatives, were as follows:

1. Antibiotics (polysporin ointment or nonpreserved chloramphenicol drops)
2. Antivirals (Vira-A ointment)
3. Mydriatic/cycloplegic (atropine ointment)
4. Glaucoma therapy (Timoptic [unit doses])

TABLE I: FIBRONECTIN STUDY GROUP FOR PHASE III

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5. Artificial tears (Refresh)
6. Steroids (Decadron ointment)
7. Steroids + antibiotics (Vasocidin ointment)

These do not contain BAK, which is thought to be detrimental to wound healing in general and may interact with FN. If this standardization involved a change in medication, the experimental treatment was not started for 1 week after such change to allow a washout period. At the end of this period, the ocular examination was repeated in order to assess any effect the change in medication may have had on the defect. If, at this time, there was evidence of healing, the patient was not entered into the study unless the ulcer became static for at least 2 weeks.

The patients continued to receive the same medical therapy that they were taking at the time of enrollment in the study during the masked portion of the study. Bandage contact lenses were not used during the masked phase of the study and for the first 2 weeks of open-label treatment.

The patients were randomized according to the etiology of the PCED:

herpes, failure to heal following surgery, thermal or chemical burn, and other. The study was a randomized, double-masked, placebo-controlled treatment with either FN or vehicle placebo with a one-way cross to FN possible either after 2 weeks in the absence of at least 50% healing or at 4 weeks in the absence of complete healing. Efficacy was defined as 100% healing while on masked therapy. To continue on masked therapy the PCED had to be 50% healed at 2 weeks or 100% healed at 4 weeks.

SCHEDULE OF OBSERVATIONS

Follow-up visits occurred at weekly intervals after initiation of treatment, at which time a complete ophthalmologic examination was performed, the degree of healing of the ulcer was assessed and documented, and any adverse effects of the treatment were noted. Photographs of the PCED were taken before and after the instillation of fluorescein. The purpose of visits at 2 and 6 weeks after the end of treatment for patients who healed was to assess the stability of the new epithelial surface.

RESULTS

Early Phase II (ie, open-label trial)

During this open-label portion of the study, 25 patients with 27 persistent corneal epithelial defects yielded 26 evaluable corneas. PCEDs were present for a mean time of 7.9 ± 11.4 weeks before FN was started. Eighteen ulcers (70%) completely healed in a mean time of 2.8 ± 2.1 weeks, four ulcers partially healed, and four did not heal. Ten ulcers completely healed (100% healing) in 2 weeks or less, representing 40% of 26 evaluable ulcers (Table II).

On the bases of these results, it was decided to conduct a double-blind placebo-controlled trial with no option for initial open-label entry, where treatment was with either FN or albumin placebo for 7 to 10 days, at which time a patient would be given open-label FN if the defect had not healed to an extent of at least 50%. Fifty percent healing as a landmark to cross over to open-label FN was thought to be a "reasonable" option that achieved two goals: trying to keep as many patients as possible in the masked (control) group while at the same time not keeping the patients on masked medication for too long a time if they were not healing.

Late Phase II (double-blind, placebo-controlled, one-way crossover)

During the double-blind, placebo-controlled portion of the study, 27 patients with 27 PCEDs yielded 24 evaluable corneas. During the masked controlled 7- to 10-day period, 12 patients received FN and 12 received

TABLE II: EARLY PHASE II (OPEN-LABEL TRIAL)*

Complete healing (mean, 2.8 ± 2.1 wks)	18/26 (70%)
Partial healing	4/26 (15%)
No healing	4/16 (15%)
Healed 100% ≤ 2 weeks	10/26 (40%)
Ever healed on FN	18/26 (70%)

*There were 25 patients with 27 PCEDs, 26 of which were evaluable. Defects were present for mean period of 7.9 ± 11.4 weeks before FN treatment was started.

albumin placebo. Among the 12 in the FN group, 7 (58%) had 50% healing or more at 7 to 10 days and continued on FN with rapid progression to total healing, while 5 (42%) had less than 50% healing and were crossed to open-label FN. Three (25%) of the 12 in the albumin group, had 50% healing or more at 7 to 10 days and continued on albumin; 9 (75%) had less than 50% healing and were crossed to open-label FN. The difference between the two groups approached statistical significance ($P = 0.1$) by chi-square analysis; for P to have reached 0.05, there would need to have been 20 patients in each group with similar responses (Table III).

Among the five ulcers originally receiving FN that were crossed to open-label FN, four (80%) totally healed with a mean time for healing of 4 weeks. Among the nine ulcers originally receiving albumin that were crossed to open-label FN, four were not evaluable and three (60%) of the remaining five healed with a mean time for healing of 1.7 weeks.

TABLE III: LATE PHASE II (MASKED DOUBLE-BLIND PLACEBO-CONTROLLED ONE-WAY CROSSOVER)*

TREATMENT	NO.	≥ 50% HEALING	HEALED < 50% (CROSSED)
Masked controlled period 7 to 10 days (initial response)			
FN	12	7 (58%)	5 (42%)
Albumin	12	3 (25%)	9 (75%)
ORIGINAL TREATMENT	**NO.**	**100% HEALED**	**MEAN TIME TO HEALING**
Open-labeled after one-way cross			
FN	5	4 (80%)	4 wk
Albumin	5/9 evaluable	3/5 (60%)	1.7 wks

*There were 27 patients with 27 PCEDs, 24 of which were evaluable. Eight (40%) of 20 ulcers ever on FN healed completely in 2 weeks or less; 14 of 17 of these ulcers (82%) healed completely at some print (3 of the 20 were lost to follow-up).

Therefore, among all the 24 ulcers, a total of 21 were ever put on FN (12 ulcers were originally on FN and 9 ulcers were crossed from albumin to FN). Among those 21 ulcers ever on FN, 20 were evaluable and 8 (40%) of them completely healed in 2 weeks or less from initiation of FN treatment. This 40% complete healing at 2 weeks correlates with the result from the open-label early phase II trial (40%). Three of four nonevaluable PCEDs were lost from the study because of surgical intervention after 2 weeks on open label, and among the remaining 17 ulcers ever on FN, 14 (82%) completely healed. This 82% is very close to the 70% total healing rate found in the open-label early phase II trial (Tables II and III).

Analyzing the first 23 patients who completely healed on FN, two groups of data were additionally compiled: Fig 1 shows the percentages of patients with 50% healing and 100% healing over time. From the results in early and late phase II, we theorize that FN is efficacious at least in enhancing the healing of PCEDs. Two questions regarding the role of topical medications in PCED arose: Does FN override the toxicity of those medications and achieve through this mechanism the enhanced healing effect, or is FN not necessary for healing if the medication toxicity itself is eliminated? Therefore, a change in the protocol was undertaken to standardize treatment with medications containing the least amount of preservatives in order to control for toxicity. A new protocol was written in which a washout period of 1 week had to precede entry into the trial when the patient was put on standard medications lacking preservatives. If substantial healing occurred on this standardized treatment, the patient was excluded from entry.

Other changes included a change of end point, where the end point for evaluation of FN efficacy became whether or not there occurred at least 100% healing of the defect during the double-masked portion of the study. The crossover time was extended to 2 weeks to preserve patients in the double-masked study group as long as possible. Thus, patients were crossed to open-label if by 2 weeks there was less than 50% healing.

To determine whether the etiology of the PCED had any influence on FN healing action, the 54 patients that were enrolled in early and late phase II were classified according to the etiology underlying the PCED (Table IV). From this information, we were able to devise four groups to evaluate (herpes, failure to heal following surgery, thermal or chemical burn, and other), and the phase III patients were randomized within these four etiologic groups to see if there was a difference in the response to healing with FN on the basis of underlying etiology.

Early Phase III (double-masked, placebo-controlled, one-way crossover, after standardized nontoxic treatment for 1 week)

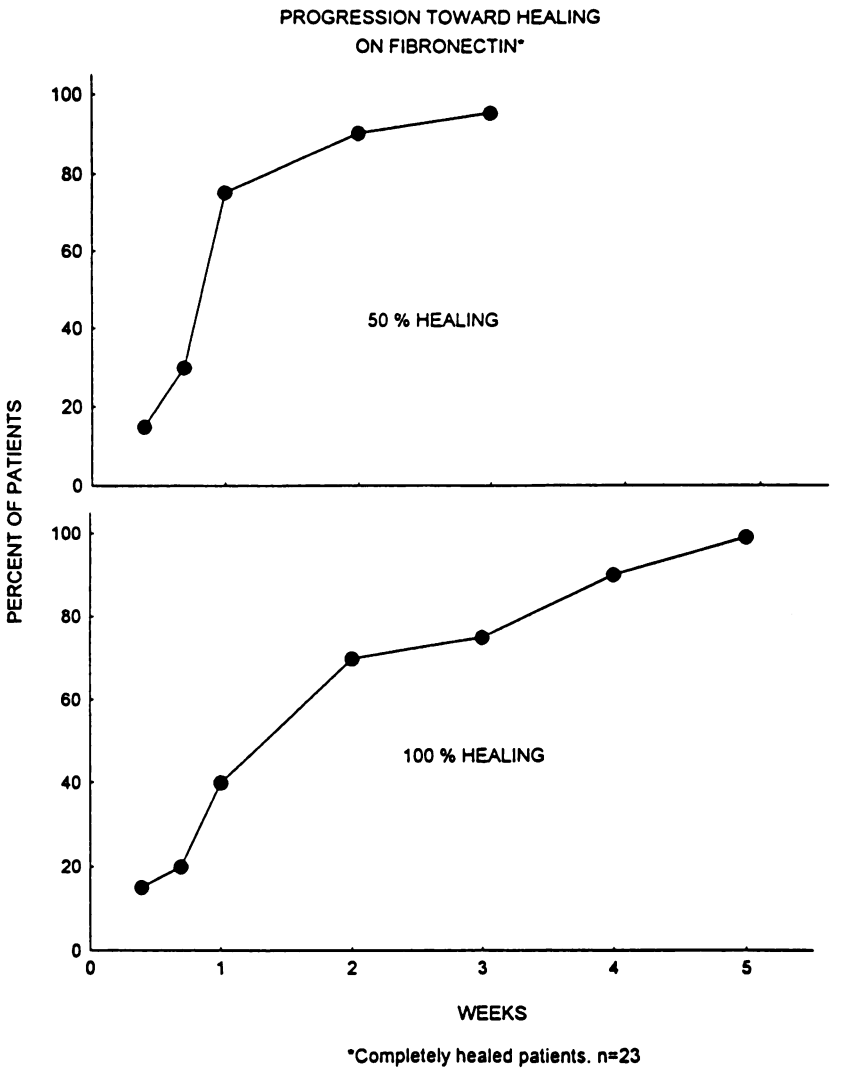

FIGURE 1
Top: Healing rate of patients who healed 50% on FN. Bottom: Healing rate of all patients who healed 100% on FN.

During early phase III, 41 patients with 41 persistent epithelial defects yielded 38 evaluable corneas. There were no statistically significant differences between the group receiving FN and the group receiving placebo with regard to the following characteristics: age, sex, race, systemic diseases,

TABLE IV: EARLY AND LATE PHASE II (ETIOLOGIES OF PCEDs)

CONDITION	NO. OF CASES
After surgery	12
Corneal burn	8
Herpes simplex	8
Herpes zoster	2
Fuchs' dystrophy	3
Other infections	3
Other	8
Unknown	10
Total	54

concomitant diseases, duration of epithelial defect, and use of prestudy medications including all kinds of ointments (except for Refresh artificial tears, which were used more by the FN patients [45.4%] than placebo patients [21.1%]). Patients enrolled in the FN group were somewhat older, more likely to be white, and less likely to have concomitant diseases. The etiologic grouping of the PCED was not statistically different between the two groups.

Only one defect in 41 (2%) healed during the initial washout period with standardized nonpreserved treatment and was excluded (Table V); this differed from the results found in a previous study.[30]

During the masked controlled initial 2-week period, 22 patients were given FN and 19 were given albumin placebo. Among the 22 ulcers receiving FN, 21 were evaluable and 12 (57%) had 50% healing or better at 2 weeks and continued on FN, while 9 (43%) had less than 50% healing and were crossed to open-label FN. Among the 19 ulcers receiving albumin, 17 were evaluable and 12 (71%) had 50% healing or better at 2 weeks and continued on albumin, while 5 (29%) had less than 50% healing and were crossed to open-label FN. There was no difference in healing between the two groups at this time (Table V).

Among the 21 evaluable corneas on masked FN, only 3 (14%) totally healed at 2 weeks and only 2 more by 4 weeks (totaling 5 at 4 weeks), with a mean time of 27 days. Among the 19 receiving albumin, 17 were evaluable, only 4 (23.5%) totally healed at 2 weeks, and 1 more by 2 weeks (totaling 5 at 4 weeks). The percentage of eyes healing 100% at 2 and 4 weeks was not higher in the FN group than in the placebo group (Table V).

Among the PCEDs originally on FN that were placed on open-label FN, 4 totally healed (with a mean time of healing of 3 weeks) and 11 did not achieve total healing. Among the 10 ulcers originally on albumin that were crossed to open-label FN, two totally healed in less than 2 weeks and 4

TABLE V: EARLY PHASE III (MASKED DOUBLE-BLIND PLACEBO-CONTROLLED ONE-WAY CROSSOVER WITH STANDARDIZED TREATMENT)*

TREATMENT	NO.	100% HEALED AT 2 WK	HEALED ≥ 50% 2 WKS AND NOT CROSSED	TOTAL PATIENTS 100% HEALED AT 4 WK
Masked controlled period				
FN	21 (22%)	3/21 (14%)	12/21 (57%)	5/12
Albumin	17 (19%)	4/17 (23.5%)	12/17 (71%)	5/12

ORIGINAL ASSIGNED TREATMENT	NO. CROSSED TO OPEN-LABEL FN	HEALED ON OPEN-LABEL FN
Open-label		
FN	15	4
Albumin	10	4

*There were 41 patients with 41 PCEDs, 38 of which were evaluable. Only one defect (2%) healed during the initial washout period. Thirteen (42%) of 31 ulcers ever receiving FN (including 10 patients who crossed from the albumin group) ever healed completely (2 patients in this group healed, then broke down; 1 of them healed again and 1 had not healed by the end of the follow-up period). Five (16%) of the 31 healed completely in 2 weeks or less. Mean time to healing was 4.1 weeks.

additional ulcers ever healed totally.

Finally, among all the 38 evaluable ulcers, a total of 32 were ever put on FN (22 ulcers were originally on FN and 10 ulcers were crossed from albumin to FN because they had less than 50% healing in 2 weeks, or 100% healing at 4 weeks). Among those 32 ulcers ever on FN, 31 were evaluable and only 5 (16%) of them completely healed in 2 weeks or less. This 16% complete healing at 2 weeks does not match the results from the early and late phase II trial. Furthermore, among the 31 ulcers ever on FN, 13 (42%) ever completely healed. This 42% complete healing if FN was ever used is different from the 70% to 82% rates found in the results from the early and late phase II trial (Tables II and III).

In comparing early phase II results with early phase III results, there was a statistically significant difference in total healing at 2 weeks and in ever healing. This was also the case comparing late phase II results with early phase III results (Table VI). This suggests that either by standardizing therapy or because of some other variable, something altered the therapeutic effect of FN, not only by slowing therapeutic effect (fewer patients completely healed in 2 weeks) but also by decreasing efficacy (fewer patients ever healed on FN).

In addition, worth noting is that 9 of 21 (42.9%) of the original FN group ever healed compared with 40% of the placebo group crossed to FN, which further indicates a consistent but lesser therapeutic effect of FN. Fig 2 shows the progression toward healing with FN in both phase II and phase

TABLE VI: COMPARISON OF PHASE II AND PHASE III RESULTS

	LATE PHASE II		CHI-SQUARE*
Healed 100% ≤ 2 wk	8/20 (40%)		4.96
	EARLY PHASE II	EARLY PHASE III	
	10/26 (40%)	5/31 (16%)	5.42
	Total 18/46 (40%)	7.55	
	LATE PHASE II		CHI-SQUARE
Ever healed	14/17 (82%)		7.29
	EARLY PHASE II	EARLY PHASE III	
	18/26 (70%)	13/31 (42%)	4.25
	Total 32/43 (75%)	7.98	

P = .05 at chi-square ≥ 3.841
P = .01 at chi-square ≥ 6.635

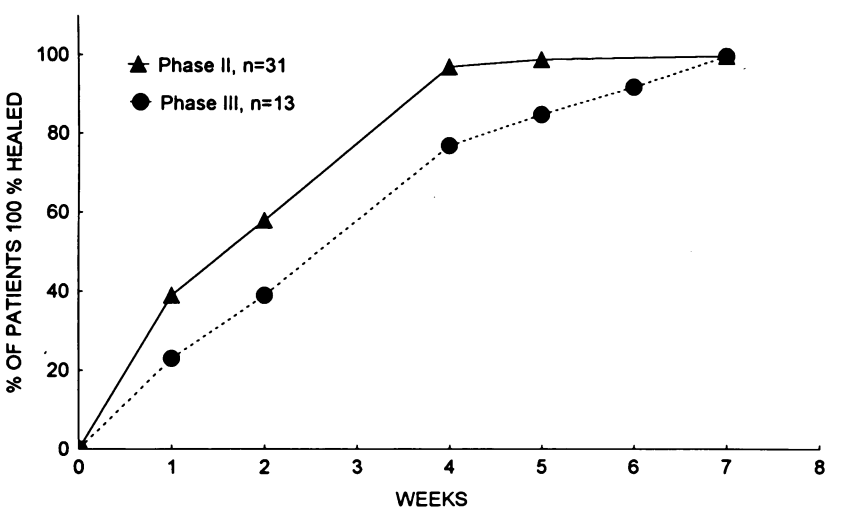

FIGURE 2
Comparison of 100% healing rate of patients in phase II and phase III. Note not only slower healing rate for those who healed, but smaller number of patients who completely healed.

III. When data from both of these phases are plotted together, the healing curves of phase III flattened compared with phase II, indicating a slower therapeutic effect of FN.

"Healing at 2 weeks" and "ever healing" was also examined by historical and clinical characteristics at the time of enrollment. These two categories were not significantly different with regard to the following characteristics: age, race, systemic diseases, concomitant therapy, duration of epithelial defect, etiology of eye disease, site of study, and use of prestudy medications, including all kinds of ointments (except for Refresh artificial tears, where healing at 2 weeks was lower [14%] when Refresh was used than when Refresh was not used (21.7%). It was considered that Refresh might have been associated with nonhealing but these differences were not seen for "ever healing." In general, "healing at 2 weeks" was more likely among females, and those who did not use Refresh, those whose etiology was burns or other; however, these changes were far from statistically significant.

Nonhealing did correlate with frequency of ointment use (Table VII). This observation is further confirmed by two facts:

1. Only 16% (5/31) of patients on FN healed at 2 weeks compared with 40% in both phase II studies, but when phase III patients were divided according to frequency of ointment use (Table VII), the percentage of patients on FN and healing at 2 weeks goes up again to 37.5% (3/8) with patients on 0 to 2 ointment applications per day (Table VII and Fig 3). This correlation was not observed when patients were on albumin (Table VII and Fig 4).

2. There was a trend of more frequent complete healing on FN if patients were on 0 to 1 ointment application per day, significant improvement on FN for those on 2 to 3 ointment applications per day, and no significant improvement on FN for those on more than three ointment applications per day (Fig 5). This trend was not observed when patients were on albumin (Fig 6). Therefore, standardized nontoxic concomitant medication consisting of more frequent application of ointment seems to interfere with the therapeutic effect of FN, and a correlation between lack of response to FN and use of ointments approached statistical significance.

DISCUSSION

There was no difference in response between the FN and placebo groups in our early phase III study, in contrast to our previous early or late phase II trials and the Japanese trials.[6,8] Furthermore, the FN group had a lower and slower response rate than in phase II or in a similar Japanese trial, which used the same FN preparation (ie, among the responders to FN, the rate of

TABLE VII: EARLY PHASE III (PERCENT HEALING AT 2 WEEKS BY FREQUENCY OF OINTMENT APPLICATION)

	0-2/DAY	3-11/DAY
FN patients	3/8 (37.5%)	0/13 (0%)
Placebo patients	2/10 (20%)	2/7 (28.6%)

	FN PATIENTS		ALBUMIN PATIENTS	
	NO.	APPLICATIONS/DAY	NO.	APPLICATIONS/DAY
Total in each group	21	3.1 ± 2.6	17	2.7 ± 2.3
Healed at 2 wks	3	0.3 ± 0.6	4	2.8 ± 2.5
Significant improvement	9	2.3 ± 1.7	7	2.6 ± 2.0
No significant improvement	9	4.4 ± 2.9	6	2.5 ± 2.2

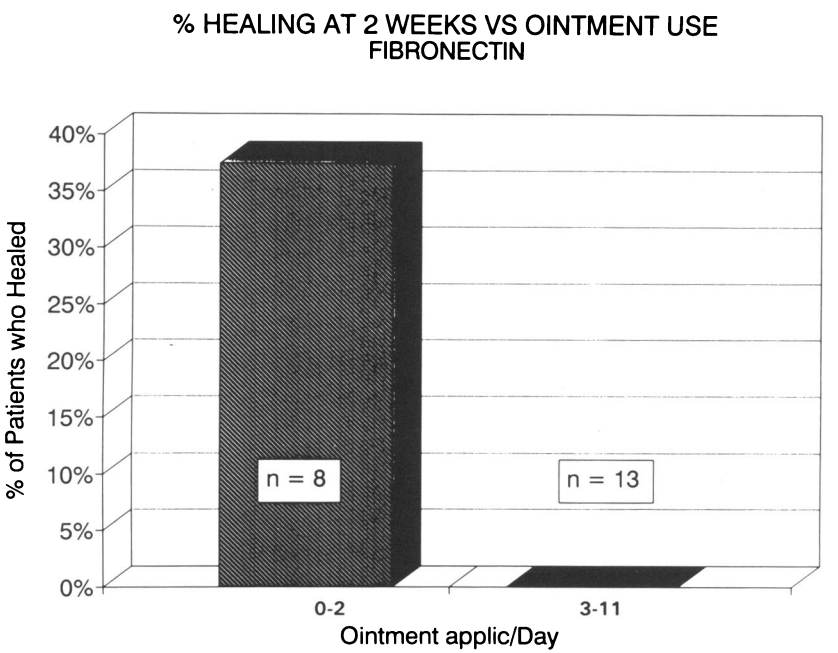

FIGURE 3
Comparison of percentage of patients on FN healed relative to number of ointment applications per day. Patients on 0 to 2 ointment applications per day had a much greater likelihood of healing than those on 3 or more applications per day.

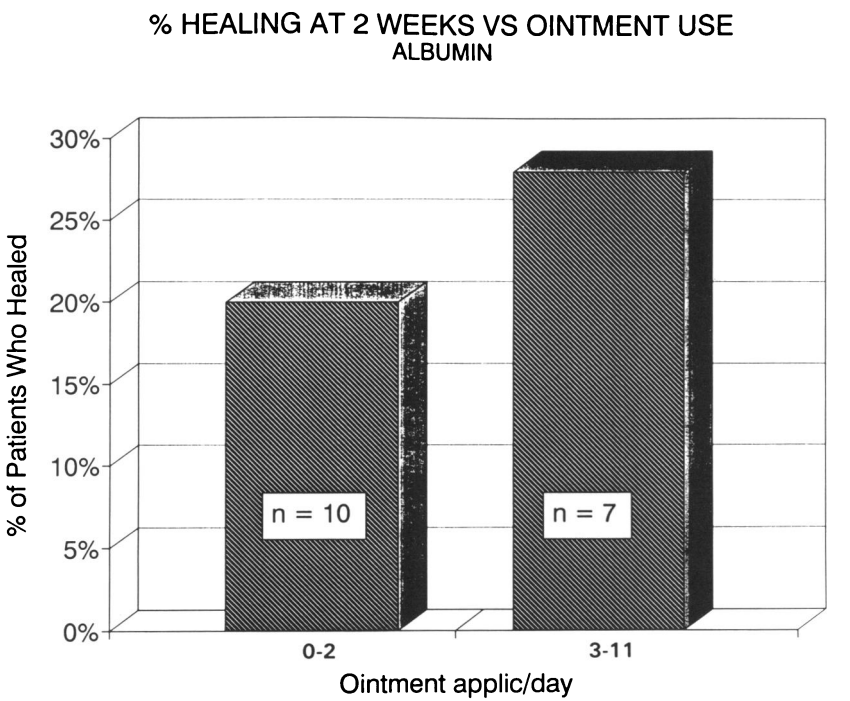

FIGURE 4
Percentage of patients who healed versus number of ointment applications. Note that there was no significant difference between healing rate in albumin-placebo group.

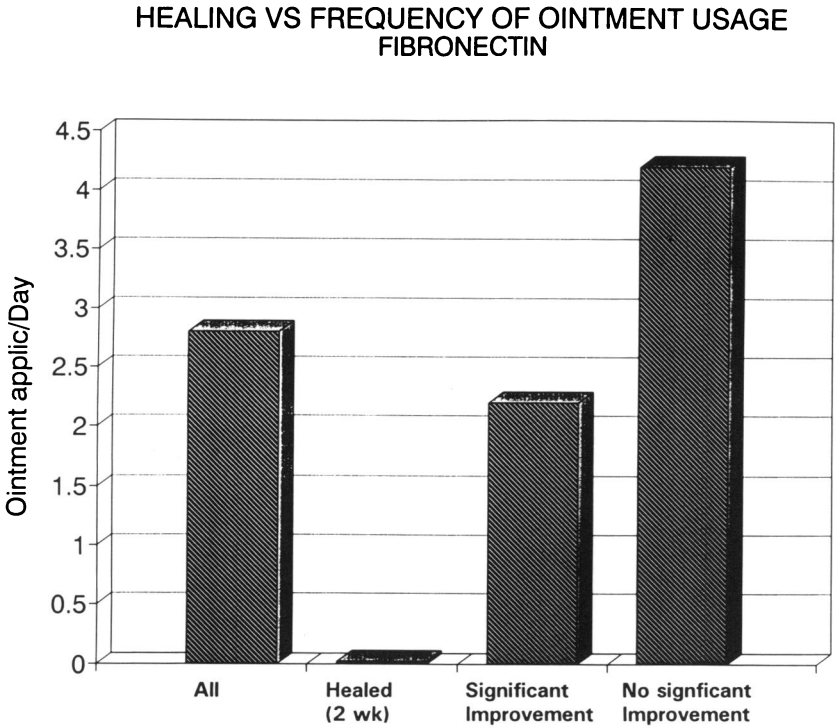

FIGURE 5

Correlation between likelihood of healing with use of FN and relatively infrequent use of ointments. Those who healed in less than 2 weeks were on 0 to 1 ointment application per day. Those with significant improvement had intermediate use of ointments (2 to 3 applications per day). Other category represents patients who showed little or no response to topical FN and who were on 4 or more ointment applications per day.

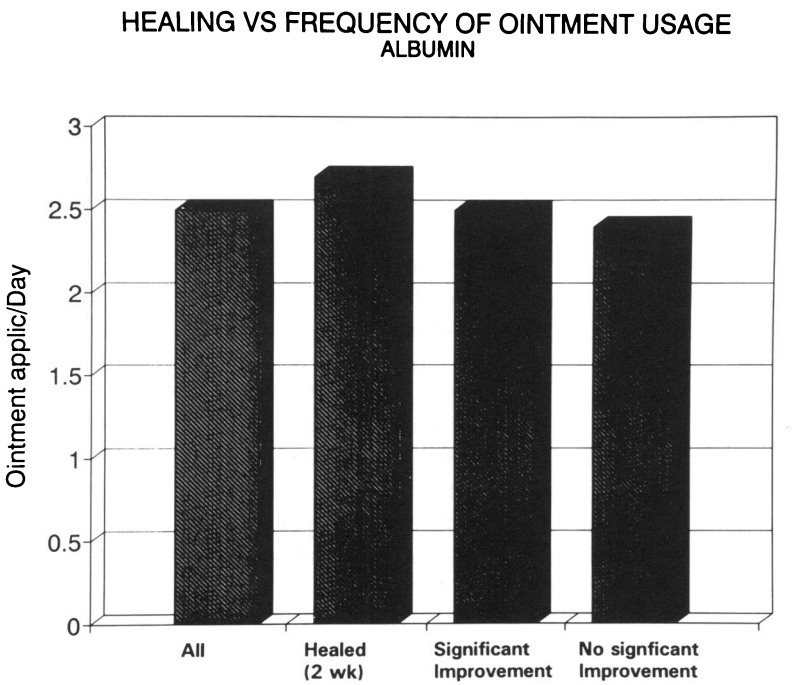

FIGURE 6
Healing rate with use of albumin placebo relative to frequency of ointment application.

healing was delayed). Multiple explanations could account for these results. By standardizing therapy and using mostly preservative-free concomitant medications, we did not alter our patient population in this study compared with phase II, because few patients were eliminated due to healing during the time of washout (2%), consisting of the use of preservative-free medications. Furthermore, FN did not seem to work by overcoming preservative toxicity, in that the albumin group response rate was unchanged from that seen in the phase II study, where preserved medications were used. The FN preparation itself did not contribute to this different result, because it was not changed from phase II to III and was similar to the one used in the Japanese study. The results were not investigator-related, because when results from the study center that carried out the phase II studies were split out and analyzed separately, they did not differ from those from the remaining centers. On the other hand, the use of newly implemented concomitant medications, in particular ointments, correlated with the reduced response to FN in a manner approaching statistical significance.

Complete healing on placebo (albumin) was 25% of PCEDs at 2 weeks, and this rate of healing was consistent in all phases (25% [3/12] in late phase II and 23.5% [4/17] in early phase III). In contradistinction, FN was associated with complete healing in 40% of PCEDs at 2 weeks, and this rate of healing showed a faster therapeutic effect than placebo and was consistent in early and late phase II (40% [10/26] in early phase II and 40% [8/20] in late phase II), and approximately 80% ever healed on FN and that rate of healing represented a higher efficacy than placebo and was consistent in early and late phase II (70% [18/26] in early phase II and 82% [14/17] in late phase II). However, when FN was used concurrently with frequent preservative-free ointments in early phase III, its efficacy in more frequent healing and in speed of healing were both inhibited. FN was associated with complete healing of 16% of PCEDs at 2 weeks; this rate of healing represents a statistically significant lesser therapeutic effect than in phase II and is similar to that seen with the albumin placebo. Only a total of 42% ever healed on FN, and this rate of healing was statistically significantly lower than in phase II.

Additionally, we can conclude that early phase III, as performed, did not conform to usual medical practice because of the extensive use of ointments; a new protocol, namely, late phase III, has been designed to deal with this flaw. The following amendments have been made to the new late phase III protocol: the FN preparation no longer contains albumin and now contains a preservative, the placebo consists of vehicle and preservative, and there is no change to standardization of medications; patients may remain on any appropriate therapy, except that ointment use is restricted to bed-

time only; cross to open-label FN occurs at 2 weeks in the absence of 25% healing or at 6 weeks if the patient was not crossed at 2 weeks and is not 100% healed. This study is now being carried out at 14 centers and should be completed within 1 year.

Analysis of all data from our phase II and early phase III studies suggests that topical FN use is safe and may be effective in the treatment of persistent corneal epithelial defects. It does not appear that therapeutic efficacy is achieved by overcoming preservative toxicity. It does appear that frequent topical ointment application interferes with the therapeutic efficacy of FN, possibly by creating a mechanical barrier and preventing access of fibronectin to the denuded stromal surface.

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DISCUSSION

DR DENIS M. O'DAY. My sympathies go out to Dr McCulley and colleagues in their struggle to define more effective treatment for PCEDs. Of all the problems one faces in the management of corneal and external disease, perhaps one of the most frustrating and difficult to understand is this condition. As their paper underscores, these lesions are multi-factorial, the etiology involving not only the primary underlying condition but also other factors, particularly the lids and their mechanical effect on the tear film, the tear film composition, the rate of tear production, the state of the corneal epithelium, the state of the underlying corneal stroma, the neurologic status of the cornea, and external factors, such as topically applied medications.

Designing a therapeutic clinical study that takes cognizance of all these factors and, at the same time, complies with FDA requirements is a nightmare. Dr McCulley's ongoing evaluation of FN as a possible useful adjuvant to corneal epithelial healing is based on a sound hypothesis, for there is good reason to believe that the endogenous FN plays a role in the complex process of epithelial wound healing. But does topically applied FN have this effect? The answer remains far from clear. The authors encountered numerous problems in endeavoring to answer this question. Part of the problem is the heterogeneous nature of the patient population presenting with epithelial defects. Another is the difficulty in ascertaining healing or progress toward healing as well as accounting for the confounding effects of other therapies

that the patient may be receiving simultaneously. The conventional wisdom is that topical pharmacologic preparations, and particularly those with preservatives, can have a profound effect on the resolution of epithelial defects. It is a common experience in the management of persistent epithelial defects that topical drug toxicity is an important variable. Withdrawal of such agents frequently leads to healing. Thus, part of the design was to try to control this variable and thus demonstrate an effect with FN. In fact, no statistically significant effect has yet been shown. However, the authors have identified a trend that suggests that concomitant nonpreserved lubricating eye ointment may, in fact, retard the therapeutic effect of FN, presumably by blocking access to the drug. If this effect can be proven, it would be an important step in understanding the apparent ineffectiveness of topical FN.

The objective evaluation of corneal epithelial would healing in a clinical setting is difficult. As already mentioned, case selection is a problem. Stratifying for the many variables is also a challenge. How to adjust for such variables as ulcer size and location is an important consideration. A method that objectively measures size and determines healing rates is a necessity. In the context of these many variables, opportunities for inadvertent bias abound, making masking of observers essential.

Dr C. Stephen Foster I would like to welcome Dr McCulley into the area of FN clinical research and Dr O'Day's lucid discussion and obviously heart felt expressions of sympathy are all too well-recognized as being appropriate by those who have done similar work.

Our group in Boston was very excited over a decade ago when we first discovered FN in the eye and in subsequent work that tried to apply FN to accelerate healing of PCEDs. This gave us some encouragement to proceed with putting together a randomized trial using placebo controls in a masked clinical trial. We were encouraged by our initial study and very discouraged by our controlled randomized masked study. Three groups in America and two in Europe have similarly failed to show a consistent statistically significant benefit of plasma FN in animal and human work. I emphasize the plasma aspect of this because it is clear that cellular FN may well have more relevance when it comes to cell attachment and cell migration than does plasma FN. I would offer to Dr McCulley two thoughts. One is that he may want to consider with his collaborators meta analysis by putting his work together with our previously published work. And he may also want to have discussions with the suppliers of his product as to whether or not there is any remote possibility that the availability of cellular FN might be a possibility in the near future.

Dr Verinder Nirankari. I think it is certainly a most frustrating experience to treat patients with persistent epithelial defects.

In previous studies, sponsored by Chiron, where they used topical FN and compared it to nonpreserved tear drops in a double-masked crossover study, they found that as soon as they eliminated previous medication used preservatives, there was prompt epithelial healing with no significant difference in healing rates between the two groups. One of the concerns of the FN study was the use of nonpreserved ointment and whether that would prevent access of the FN molecules to the

epithelium. However, in that study (personal communications) the ointment was used only at bedtime, and therefore should not have significantly affected uptake of FN by the epithelium.

I would like to ask Dr McCulley, that knowing the complex pathways to epithelial healing, whether he has considered other molecules that promote epithelial healing such as EGF and whether that may have an additive beneficial effect over the use of FN alone.

DR RICHARD FORSTER. I was impressed with the longevity and persistence of the epithelial defects in your initial open study. An average of 8 weeks suggests factors other than epithelial healing that had contributed to the persistence of the defect. I also noted the absence of a description of the function of the lids and other factors that may be contributing to the persistence of these defects. I am impressed that when a defect lasts more than a couple of weeks there is usually a noncorneal cause for the defect, usually inflammatory limbal conjunctival or lid disease. I would ask whether in your future planned studies if you think it would be relevant to perhaps recategorize the persistent defects as regard associated lid conjunctival and limbal disease.

DR JAMES MCCULLEY. I want to thank you for your comments and Dr O'Day thank you for your very kind and even handed discussion.

The persistent epithelial defects indeed are a heterogeneous population. And the underlying disease may very well play a role in the response or the lack of response to FN. That is one of the reasons that we tried in our last study to randomize within the four different underlying diagnostic groups. We just simply don't have enough information yet, if FN does work, to determine which patients are going to respond and which are not. It is hard to predict at this time. I hope if, indeed, it does prove to be efficacious that we will gain enough information so that we can predict before the fact which patients are apt to respond and which patients are not. Relative to the comment about the ointment, Friedenwald years ago published a paper that showed that ointment application impeded epithelial healing. How the ointments played a role in this study, we are not certain. We have asked our Japanese colleagues who have shown positive results in patients to look in their animal model to see if they can try to determine the role of ointments if, indeed, there is a role. Our very simplistic attitude about this is, when one puts a lot of ointment on the eye that FN cannot gain access to the surface. Well, maybe that is too common sensical to prove to be true in modern biology, but that is at least the thought right now. In looking at the placebo-controlled masked study, it is a lot easier to watch a patient long-term with a potentially blinding problem if one feels like one is doing everything one can do for the patient. So an open-label study is much easier to do than a double-masked study. One of the problems with the studies is one has to reach statistical significance in comparing drug to placebo. Therefore, the study must be designed so that one can maintain masked therapy as long as possible. So how is one going to devise a study where one can maintain the control group? It is difficult to maintain a masked-placebo controlled study when patients have a progressive potentially blinding

disease, when one does not know with what the patients are being treated. This concern led to our current approach wherein if there is less than 25% response at 2 weeks, patients can be switched to open-label. We hope that with this approach we will be able to maintain enough patients that are in the masked phase so that if there is a therapeutic response to FN we will have a large enough group with which to compare. If we do not maintain those patients, which I do not think any other studies have effectively done, we cannot reach statistical significance because we will be comparing FN response to nothing.

Our FN is plasma derived. It is hoped that in time, we will have available nonplasma derived FN that will be genetically engineered.

The Chiron study did not show therapeutic efficacy for reasons that are not clear. They did have approximately 60% of their patients heal when placed on non-preserved therapy. That clearly was not the case in our study. That is not necessarily the only variable, so I am not sure of the final analysis of that and why their study is different. It was clearly very different. They have only published their study in abstract form. The complete data have not been published on their applying topical FN in PCEDs. They have published their negative result with its use of FN in dry eyes.

To expand on what I said before about how Chiron study relates to their preparation: the Japanese have shown that 1 mg % is the optimal dose concentration for therapeutic response; response declines as concentrations increase above that level. Three milligrams percent, as used in the Chiron study may be too much of a good thing. There was a preservative of chlorbutanol which may have been interfering. They also sterilized the preparation with heat. If one heats a solution with proteins one can alter them. I am not sure that they indeed had a biologically active intact molecule in their preparation. We know that we do in ours. This has been shown and has been published.

Adding a growth factor of EGF to FN, I doubt will occur very soon. Chiron evaluated EGF and had disappointing results. I would like to have it available in the future for further study, but the only way we would know if a growth factor was efficacious would be to test it when it is the only unknown variable. One cannot add it to something like FN and try to figure out which of the two is effective or if there is independent or added efficacy. So I think we have to establish first the efficacy or lack thereof of FN and then we would have to look at EGF alone and then potentially in combination with other molecules. And, yes I am persistent and we still do not have the answer. I am still not sure what the answer is, but we are going to stay after it, I hope, until we get this settled one way or another. I hope with this current study in which we have approximately half the number of patients enrolled that are needed to answer the question, we will finally be able to say yes, FN is therapeutically advantageous or it is not. And we may gain some insights into which patients will respond and which will not.

Patients with lid abnormalities contributing to their persistent epithelial defects had the lids repaired first, but continued with persistent epithelial defects. This was not a confounding variable in the study. So a person with an entropion with persistent epithelial defect was not involved in the study unless the entropion was

repaired and the defect did not resolve. So I do not think we have that as a confounding factor.

I thank all of you for your comments and thank you for the opportunity to participate.