Interferon Alfa-2b Suppresses Proliferative Scar (Hypertrophic Scar and Keloid) Fibroblast Contraction *in Vitro*

Keiichiro SAHARA and Takahiko MORIGUCHI

Department of Plastic and Reconstructive Surgery, Kawasaki Medical School, Kurashiki 701-01, Japan

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ABSTRACT. Purpose : An in vitro model of dermis was reconstituted by introducing fibroblasts on type I collagen gel. The model was maintained in culture to investigate the cellular behaviors of human dermal fibroblasts and proliferative scar fibroblasts. Increased fibroblast activity and collagen production have been observed frequently in proliferative scars. Previous studies have demonstrated that interferons suppress collagen production by means of normal, hypertrophic scars, and keloid-derived fibroblasts. We used the FPCL to evaluate the effect of interferon on fibroblasts harvested from normal human skin, human hypertrophic scars, and keloid tissues. Methods : Human recombinant interferon alfa-2b (IFNα-2b : 1000 IU/ml) was added to the culture media. The collagen gel was overlaid with 1×10^5 fibroblast cells. Results : The keloid FPCL showed the highest contraction. Contraction in all the groups appeared to be suppressed by IFNα-2b during the first 72 hours of the experiment ($p<0.05$). Conclusion : The contractile properties of fibroblasts taken from normal human skin, hypertrophic scars, and keloids in this in vitro study were suppressed by IFNα-2b. The contraction obtained in the interferon-treated keloid group was similar to the contraction obtained in the untreated normal skin. These findings suggest that IFNα-2b may be beneficial for the treatment of proliferative scars.

Key words : FPCL — hypertrophic scar — keloid — IFNα-2b

Fibrosis is a normal reparative process that occurs after tissue injury. It is characterized histologically by fibroblast hyperplasia and increased collagen deposition. In addition, contraction of the fibrotic mass may occur and may be required for normal wound healing. Contraction can also lead to distortion of normal tissue architecture and contribute to organ dysfunction in pathologic fibrosis.[1] After tissue injury, resident fibroblasts undergo a metabolic activation and exhibit increased growth and synthetic rates to repair injured tissue. Activation likely occurs when resident fibroblasts are exposed to fibroblast-stimulatory factors/cytokines during the early inflammation-associated phase of the fibrotic response.[2-4]

Hypertrophic scars and keloid tissues are examples of fibroblast proliferation and overabundant collagen deposition in healed wounds.[5] Collagen deposition has not been shown to be the result of decreased collagenase activity in keloids.[6,7] Instead, it has been suggested that the effectiveness of collagenase activity may be diminished. The biological and clinical differences between hypertrophic scars and keloids have still not been clearly established.[8,9] Currently, there is no safe,

佐原慶一郎，森口隆彦

systemic, preventive treatment for regulating fibroblast proliferation, collagen production, and scar contraction in proliferative diseases.

Interferons are important soluble products of cells involved in inflammatory reactions and are capable of influencing the proliferation of various fibroblastic cell lines.[10–13] Interferon alfa-2b (IFNα-2b) inhibits the proliferation and certain biosynthetic activities of fibroblasts derived from a variety of tumor and transformed cell lines, as well as from normal skin.[10,13] Investigators have suggested that interferons may play an important role in the regulation and, possibly, the termination of fibrosis by suppressing persistently activated fibroblast functions.[11,12]

There are no satisfactory *in vivo* systems available for the study of proliferative scars. The observation that fibroblasts that are cultured in collagen gel cause the gel to has contract made it possible to study fibroblast behavior in a well-controlled environment.[14,15] This *in vitro* fibroblast-populated collagen lattice (FPCL) system has been used for wound healing studies and has been prepared as a possible replacement for damaged skin.[16] The ability of fibroblasts to contract collagen gels is one of the specific fibroblast functions that might also occur *in vivo*. Some authors[17] consider studies made in FPCLs to be more physiologic than those made on monolayer cultures.

In this study with the FPCL model, we evaluated the effect of IFNα-2b on fibroblasts harvested from normal skin, hypertrophic scars, and keloids.

MATERIALS AND METHODS

Materials and culture conditions

Primary fibroblast cultures were established from six hypertrophic scars, four keloids, and eight normal skin samples obtained by surgical procedures (Table 1). The specimens were sectioned into 1 to 2 mm segments, placed into tissue flasks with Dulbecco's modified Eagle's medium (DMEM) and penicillin (100 IU/ml ;

TABLE 1. Cases of proliferative scars

Case	Age · Sex		Scar	Site	Cause	Passage
1	14y	M	HTS	abdomen	ope.	2y5m
2	21y	F	HTS	knee	trauma	1y2m
3	29y	F	HTS	abdomen	ope.	1y8m
4	10y	M	HTS	chest	trauma	3y5m
5	49y	M	HTS	elbow	trauma	1y5m
6	36y	F	HTS	abdomen	ope.	2y2m
7	38y	F	KL	shoulder	trauma	10y5m
8	37y	F	KL	abdomen	ope.	8y5m
9	25y	F	KL	ear	pierce	2y5m
10	24y	F	KL	abdomen	ope.	5y5m

HTS : Hypertrophic scar
KL : Keloid
ope : operation
passage : period from injury to scar revision
y : year, m : month

Gibco Labo, Grand Island, NY, USA), streptomycin (100 μg/ml ; Gibco), and 10% fetal bovine serum (FBS ; HyClone Labo, Logan, Utah, USA). Then they were incubated at 37℃ in a humidified atmosphere of 5% carbon dioxide. The culture medium was renewed every three days. Confluent primary cultures were trypsinized and subcultured. The cell cultures were studied during passages five through seven.

Interferon treatment

Subconfluent fibroblast cultures were arranged by placing 1×10^4 freshly trypsinized fibroblasts, contained in 2 ml of DMEM with 10% FBS, into triplicate wells with or without IFNα-2b (Schering Co, Kenilworth, NJ, USA). Human recombinant IFNα-2b (1000 IU/ml) was present in the culture media for 14 days until the confluence was reached. The culture medium was renewed every three days with or without IFN.

Preparation of collagen lattice

The collagen lattice was prepared as previously described with Type I collagen (derived from porcine tendon, Nitta Gelatine Co, Tokyo, Japan).[14,18,19] Then, 1 ml of this mixture was poured into a 35-mm bacteriological dish (Falcon Co, Kanagawa, Japan). The final collagen concentration was 1.5 mg/ml.

Measurement of gel contraction

The cells were collected by trypsinization and centrifugation at day 14 and resuspended in culture medium. Cell numbers were adjusted to 1×10^5 cells/ml. Then we overlaid 1 ml of this solution on the collagen gels and incubated it at 37℃ in a humidified atmosphere of 5% carbon dioxide.

The amount of gel contraction was measured at 4, 8, 12, 24, 48, and 72 hours after incubation. Acetate overlays were used for tracing the perimeters of the gels. The traced areas were digitally measured with the aid of a personal computer (Apple Japan, Inc, Tokyo, Japan).

Statistical analysis

Repeated collagen gel area measurements were transformed to reflect the percentage of remaining area over time. These data underwent analysis of variance ; the significance of the differences among the overall group means was tested by Duncan's range test ($p<0.05$).

RESULTS

FPCLs containing normal human skin, hypertrophic scar, and keloid fibroblasts showed continuous reduction in gel areas for 72 hours (Fig 1). The overall group means differed significantly among the untreated groups ($p<0.05$).

Reduction of the gel area was highest in the keloid group. FPCLs containing keloid fibroblasts contracted to 32.1%$\pm$3.8% of the original area in 24 hours and 28.3%$\pm$3.6% in 72 hours (67.9% and 71.7% contraction, respectively). FPCLs containing hypertrophic scars and normal skin fibroblasts contracted to 39.0%$\pm$1.9% and 48.1%$\pm$2.8% of the original areas in 24 hours (61.0% and 51.9% contraction), and 33.4%$\pm$4.7% and 40.3%$\pm$5.0% of the original areas in 72 hours (66.6% and 59.7% contraction).

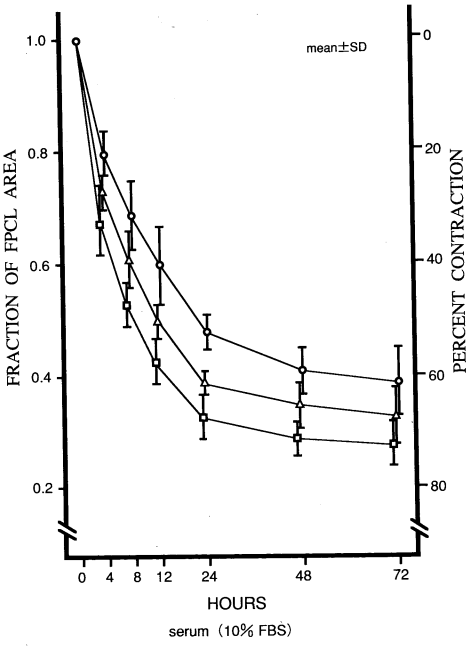

Fig 1. Contraction of collagen lattices by human dermal fibroblasts (○) and fibroblasts isolared from hypertrophic scar (△) and keloid (□)
Contraction curves were significantly different ($p<0.05$).
FPCL: Fibroblast-populated collagen lattice

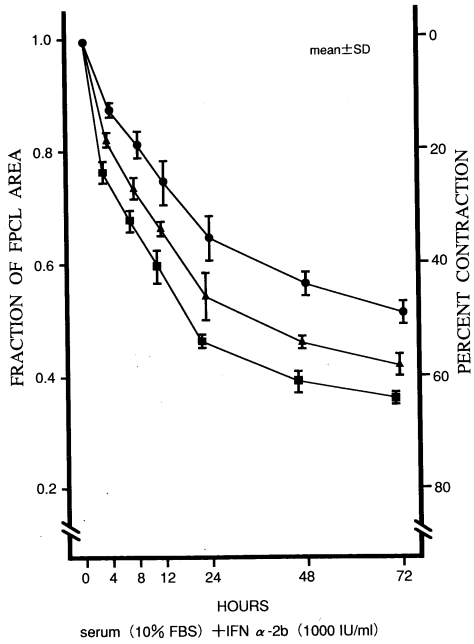

Fig 2. Contraction of collagen lattices by all IFN-treated groups, human dermal fibroblasts (●), hypertrophic scar derived fibroblasts (▲), and keloid derived fibroblasts (■)
Contraction curves were significantly different among IFN-treated groups ($p<0.05$).
FPCL: Fibroblast-populated collagen lattice

Treatment of fibroblasts with IFN α-2b reduced contraction in all the groups by 9% to 17% (Fig 2). FPCLs containing keloid fibroblasts contracted to 48.0%±1.0% of the original area in 24 hours and 37.4%±0.7% in 72 hours (52.0% and 62.6% contraction, respectively). FPCLs containing hypertrophic scars and normal skin fibroblasts contracted to 55.1%±3.6% and 65.3%±3.7% of the original areas in 24 hours (44.9% and 34.7%), 43.1%±1.9% and 51.4%±2.0% in 72 hours (56.9% and 48.6% contraction). The contraction curves were also differed significantly among the interferon-treated groups ($p<0.05$). However, there was no significant difference in the reduction of contraction by interferon among the groups (Fig 3).

The contraction obtained in the interferon-treated keloid group did not differ from that obtained in the untreated normal skin FPCL group (Fig 4). The overall mean collagen gel areas for these groups were similar.

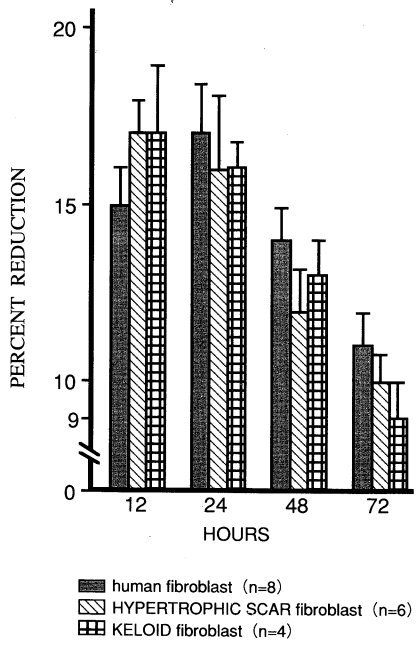

Fig 3. IFN-induced reduction of FPCL contraction was not significant among groups ($p>0.05$).
FPCL: Fibroblast-populated collagen lattice

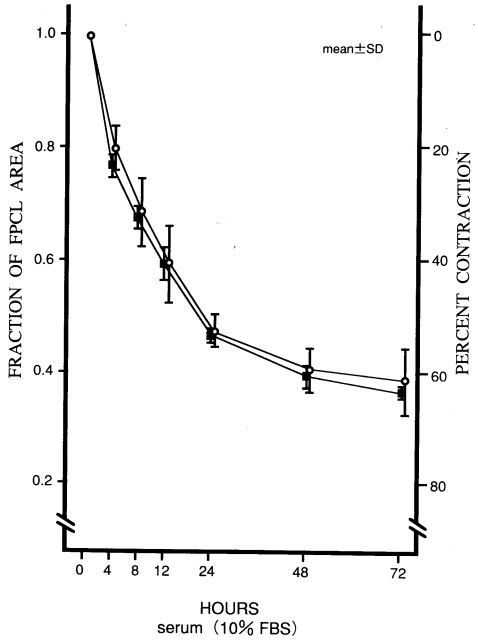

Fig 4. IFN-treated (IFN α-2b, 1000 IU/ml) keloid fibroblast (■) showed similar contraction with normal human dermal fibroblast group (○), ($p<0.05$).

DISCUSSION

Abnormal fibrosis is not unique to keloid formation and is the predominant pathologic change in other serious cutaneous and systemic diseases, including scleroderma, chronic graft-versus-host disease, pulmonary fibrosis, and liver cirrhosis. Excessive deposition of collagen is a major pathologic feature in several fibrotic diseases that can affect the skin or a variety of internal organs. In many of these conditions, an increase in collagen synthesis may not be the primary event of the disease process. Nevertheless, the excessive accumulation of collagen has major consequences in terms of the structure and function of the affected organs.[20] Fibroblasts are responsible for the production and maintenance of the connective tissue matrix by synthesis and degradation of its components. This balance of synthesis and degradation is only transiently altered during normal repair processes, as it is in wound healing, but abnormalities in matrix component metabolism may be responsible for the excessive fibrosis that occurs in keloids and hypertrophic scars.[13,21] The overabundant deposition of collagen in keloids may result from decreased collagen degradation. Yet, collagenase activity has been reported to be normal[6,7] or even increased.[8] In addition, collagen degradation has been reported to be normal in tissue explants[6] and keloid fibroblast cultures.[9] Thus, it has been suggested that a pharmacologic approach that could arrest collagen deposition in the tissues would be beneficial to patients with clinical fibrotic diseases.[22–24]

In our study, the untreated keloid FPCL showed the greatest contraction as compared with both hypertrophic scar and normal skin control groups. Interferon treatment suppressed keloid, hypertrophic scar, and normal skin FPCL contractions. The suppression of the contractions was similar in all the groups. The extent of contraction obtained in the interferon-treated keloid group resembled that of the untreated normal skin FPCL contraction. These findings suggest that interferon normalizes the behavior of keloid fibroblasts in an FPCL system.

The mechanism of fibroblast-mediated collagen gel contraction is poorly understood and is still controversial.[25] Previous studies on the contraction of collagen gels have led to the suggestion that this process approximates cell-matrix interactions *in vivo* and may be an important mechanism of connective tissue morphogenesis.[14,26–35] Gillery *et al*[32] suggested that the contraction of collagen lattices by human skin fibroblasts appears is a complex phenomenon that requires at least two conditions : the presence of macromolecular serum components and protein synthesis by the cells.[32] Although interferons are only transient inhibitors of normal fibroblast functions, they do act as persistent deactivators of at least one activated fibroblast function.[11] It is known that protein synthesis is inhibited by interferon in culture.[10] We do not know whether the effect of interferon in our experiment was due to persistent suppression of protein synthesis because protein synthesis was not measured in the FPCL. In addition, contraction of the *in vitro* collagen matrix is related to the movement of fibroblasts in the gel, not to renewed collagen synthesis. It is possible that the synthesis of fibronectin, which probably aids fibroblast-mediated collagen gel contraction,[25] is reduced by the action of interferon.

Abnormal scar contraction is one of the factors in progression of the fibrotic process. Because contraction is suppressed in the FPCL by interferon, this may be considered to be a favorable effect. Keloids and hypertrophic scars may benefit from interferon treatment, regardless of the mechanisms leading to aberrant fibroblast activation. It is noteworthy that the reduction of contraction in each group was similarly affected by interferon. This finding also supports the concept of general *in vivo* fibroblast deactivation by interferons, as has been reported in previous studies.[11,36,37]

In conclusion, our findings support the potential role for IFNα-2b as a down-regulatory growth factor for the treatment of proliferative scars. Findings also suggest that IFNα-2b may delay wound healing. It is possible that this *in vitro* model system can be used to determine the mechanism of this regulatory effect on wound repair. Further studies involving the investigation of the keloid fibroblasts of additional patients are necessary.

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