

2002 6

가

	1
I.	3
II.	6
1.	6
2.	7
3.	8
가.	8
. MTT assay	8
.	8
. MMP assay	9
4.	10
III.	11
1.	11
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3. 1 mRNA RT-PCR	14
4. MMP-2	15
IV.	17
V.	23
	24
	28

1.		7
2.	MTT	13
1.		12
2.	MTT	14
3.			
	1	mRNA
			15
4.			1
	mRNA	16
5.	Gelatin gel	zymogram
			16

가 가

가

(heparin sulfates)

, matrix metalloproteinase(MMP)

가 tissue inhibitors of metalloproteinase -1(TIMP-1)

, endothelial

cell growth factor(ECGF) 가

(Interferon -),

(interferon -)

tumor necrosis factor -

(TNF -)

T 가

가

collagenase

가

가

collagenase

MTP

가 . (reverse

transcription -polymerase chain reaction, RT -PCR)

1 mRNA

zymogram

MMP -2 가 .

1 mRNA MMP -2

가 . 가

- : , ,

< >

I.

(collagen)

, .
가
,
가 가 가 .
, ,
가 가 .
Kirsher (fibrin) thrombin
(myofibroblast)
가 , fibronectin
ischemia ¹ mechanical theory²,

³, 가 ⁴, ⁵ 가
 .
 , 가(type III/type I collagen
 가), collagenases(matrix metalloproteinases -1; MMP -1) , tissue
 inhibitors of metalloproteinase(TIMPs; α 2 -macroglobulin, α 1 -antitrypsin) 가
^{6,7} .
 (in vitro)
 (triamcinolone acetonide) glycosaminoglycan
 , , plasma protease inhibitors
 collagenase ^{6,12} .
 transforming growth factor - β (TGF - β) insulin -like growth
 factor -1(IGF -1)
⁷ . ,
 , MMP -1 가 TIMP -1
¹⁵ .
 , endothelial cell growth
 factor(EGGF) 가 가 ¹⁶ .
 .
 (Interferon -), (interferon -) tumor necrosis factor - (TNF -
) T 가
 .
 가 collagenase 가

6.7

가

II.

1.

5

(1).

Waymouth

Petri dish

phosphate -buffered saline(PBS; Sigma Chemical Co.,

St. Louis, Mo., USA)

2

37 °C 0.25%

trypsin(Sigma Co., USA)

7ml 가

Pasteur pipette

10

penicillin(300units/ml),

streptomycin(300µg/ml), actinomycin(25µg/ml)

10% fetal

bovine serum(Gibco BRL, Grand Island, NY, USA)

Dulbecco's modified

Eagle's medium(DMEM; Gibco BRL, Grand Island, NY, USA)

100ml 가

3500RPM

15

20

5ml

CO₂

(Forma, USA)

95%

, 5%

CO₂, 37°C

3

2-3

가

confluent state가

PBS

0.025% trypsin 2ml 가

5

hemocytometer

2x10⁵

24

5

1.

6	F
27	M
40	F
44	M
24	F

2.

1() : 5

2 : 24

100U/ml(100uM) 가 5

3 : 24 500 µg/ml 가 5

4 : 1:1

5 : 1:2

6 : 2:1

7 : 24 100U/ml 가

5

3.

가.

5

. MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay

MTT Assay . 96 well plate
, fibroblast 1mg/ml
MTT(Sigma Chemical Co., St. Louis, Mo., USA) , 37 °C 4
, MTT가
Dimethylsulfoxide(DMSO) MTT
formazan , spectrophotometer(DU -65, Beckman Coulter, Fullerton,
Calif., USA) MTT .

① Total RNA

RNeasy Kit[®] (Qiagen, Hilden, Germany)

RNA

② DNA(complementary DNA; cDNA)

RNA 7 µg 10mM dNTP 2 µl, oligo dT primer 10pmol,
RNase inhibitor, 1 µl reverse transcription buffer (250mM Tris-HCl pH 8.3,
375mM KCl, 15mM MgCl₂ 50mM DTT), MMLV reverse transcriptase 1 µl
volume 25 µl 70 5 RNA

(denaturation) 37 60 cDNA

95 5

③ PCR 1 cDNA

1 cDNA sequence(5'-

ATGTTTCAGCTTTGTGGACCTCC -3'; 5'-CAATCCTCGAGCACCCCTAGA -3')

(primer) 1 cDNA

RNA cDNA (3 μ) 10mM dNTP primer

1 μ volume 25 μ PCR RNA

RT-PCR -action

primer PCR PCR

. Matrix metalloproteinase(MMP) assay

Zymogram SDS-PAGE(10% acrylamide) MMPs가

gelatin . Separation gel 30% acrylamide:bis stock

3.3ml, 1M Tris-HCl(pH 8.8) 2.5ml, 10% SDS 0.1ml, 2% gelatin 1ml, 3ml

10% ammonium persulfate 0.1ml TEMED 4 μ 가

Stacking gel 30% acrylamide:bis stock 0.83ml, 1M Tris-HCl(pH 8.8) 0.63ml,

10% SDS 0.05ml, 3.4ml 10% ammonium persulfate 0.05ml

TEMED 5 μ 가 . Reservoir buffer

2.5% Triton X-100(Sigma, St. Louis, MO, U.S.A.) 100ml

1 SDS , 가

III.

1.

2-3

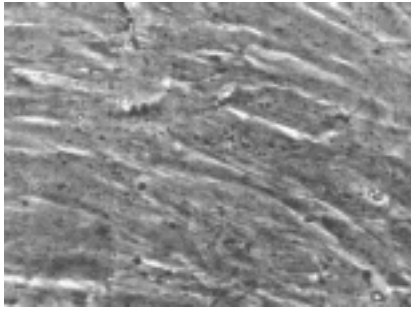
가

1

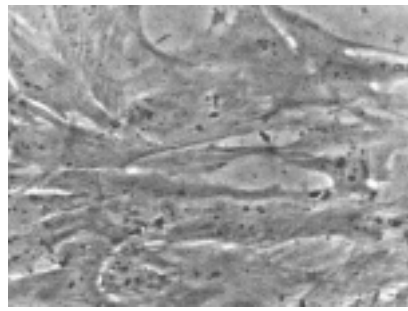
가

(cell rounding)

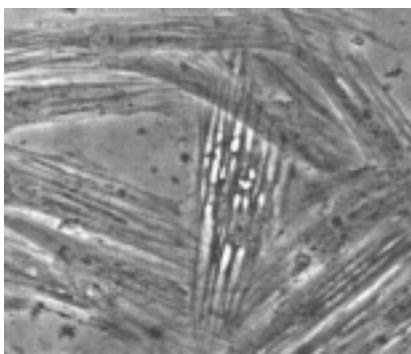
. (.1)



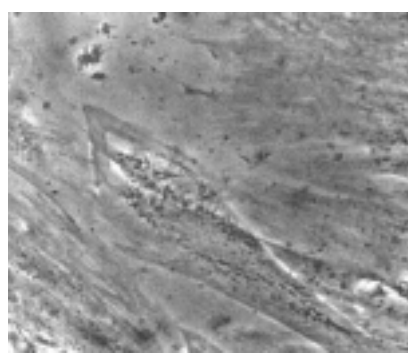
1()



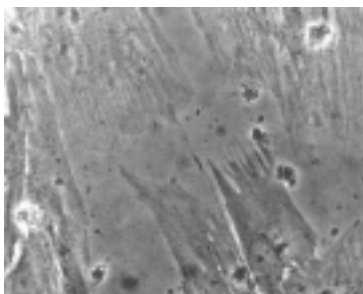
2



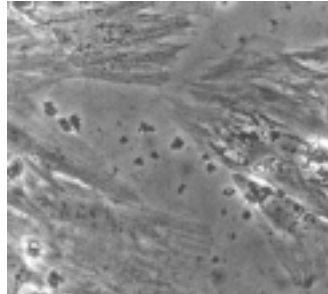
3



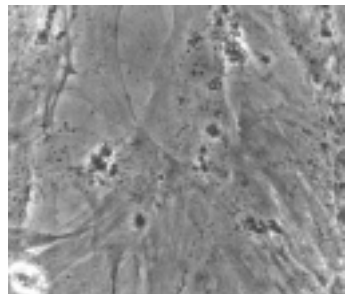
4



5



6



7

1. .

2 7 가

.

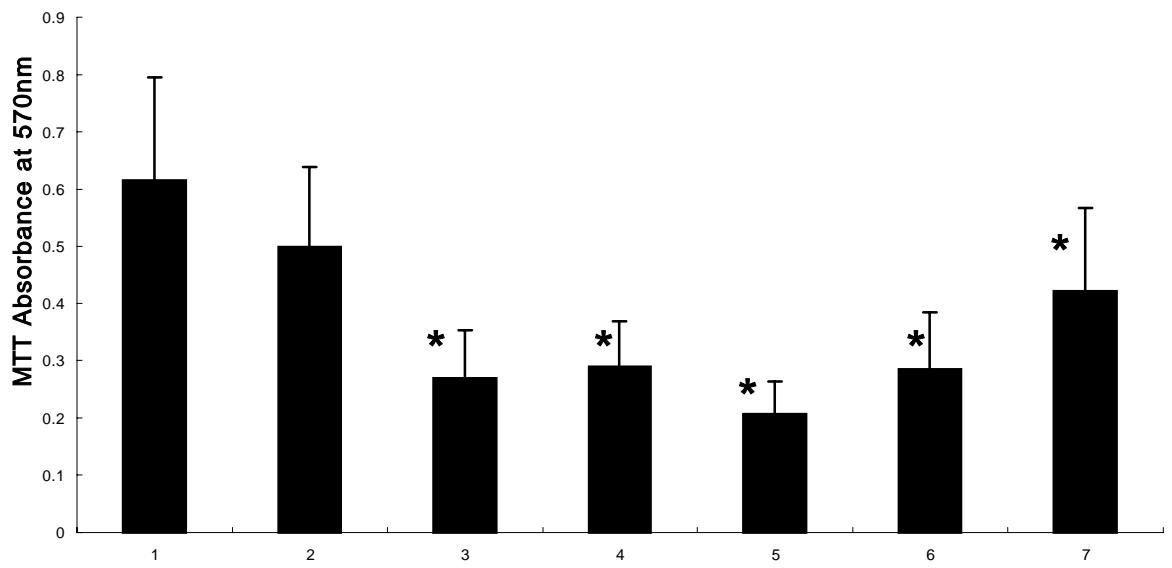
2. MTT assay

MTT (0.616 ± 0.179) (p<0.05).

가
 가 (p<0.001). 3()
 4-6() 가
 가
 (p<0.05),

		TA	HS	TA+HA(1:1)	TA+HA(1:2)	TA+HA(2:1)	IFN-
1	0.558	0.453	0.346	0.281	0.206	0.253	0.265
	0.28	0.255	0.189	0.167	0.135	0.17	0.253
2	0.574	0.683	0.311	0.325	0.288	0.344	0.453
	0.524	0.623	0.294	0.282	0.267	0.317	0.475
3	0.899	0.646	0.383	0.41	0.236	0.449	0.620
	0.902	0.619	0.373	0.401	0.22	0.426	0.581
4	0.598	0.487	0.167	0.272	0.231	0.267	0.302
	0.603	0.461	0.164	0.239	0.221	0.245	0.237
5	0.627	0.393	0.239	0.26	0.149	0.221	0.541
	0.591	0.373	0.226	0.254	0.113	0.157	0.493
	0.616	0.450	0.270	0.289	0.207	0.285	0.409
	±0.179	±0.140	±0.083	±0.079	±0.057	±0.099	±0.178

2. MTT (TA: , HA:
 , IFN- :).
 MTT 가 2



2. MTT (* : 1
 가) (1)
 MTT 가
 2

3. 1 mRNA RT-PCR .
 가 3,4,5,6 β-actin
 1 mRNA
 (3). 7() 1 mRNA
 .(4)

4. MMP -2

(1) 가

MMP -2(72Kd 68Kd) 가

MMP -2가 가 .(5)

3.

1

mRNA . 가

β -actin mRNA

1 mRNA 가

5

1

mRNA .

1 2 3 4 5 6 7



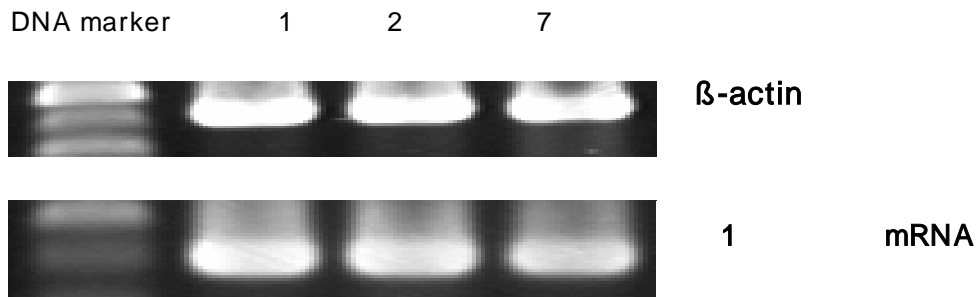
β -actin



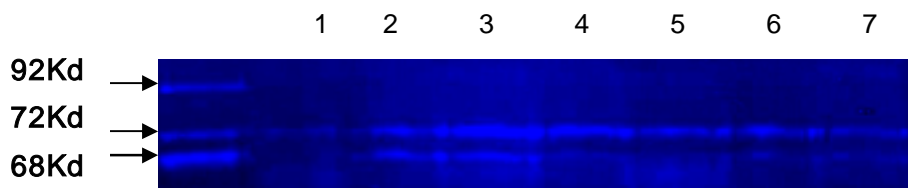
1

mRNA

4. 1 mRNA
 7 (1) 1 mRNA



5. Gelatin gel zymogram . MMP -2(72Kd, 68Kd)
 . MMP-2 가 .



IV.

6,7 .
가
8 , 7,9,10
가 가
in vitro , fibronectin, elastin
proteoglycans 6,7 glucocorticoids¹²,
10,11,13 , phorbol esters¹⁴ metabolic
TIMPs(α 2-macroglobulin, α 1-antitrypsin)가
collagenase , 가
6,7 .
가 .
TGF- β 가 TGF- β
가
6,7 . TGF- β

cytokine , fibronectin, proteoglycan

collagenase protease

inhibitors(TIMPs) 가 7,11 .

가 protease 가 TGF - β

가 . TGF - β

가

cytoskeletal

actin filaments depolymerization

cytoskeleton collagenase 가

가 17,18 . 가

(cell rounding) collagenase 가 TGF - β 1

TIMP -I 가 17 . procollagenase

. Cell rounding

cytochalasin B

, Lee (1996) 가 actin

filaments depolymerization procollagenase

가 18 . 가

collagenase TGF - β 1

가 .

β -actin

1 mRNA

zymogram MMP-2가 가

가

Tyagi¹⁵ d MMPs

mRNA protein 가 TIMP-1

, ECGF 가

¹⁶

MMPs 가

가

1 mRNA MMP-2가 가

가

-smooth muscle actin

in vitro in vivo 1 3

mRNA ²². Collagenase

가 ⁶ ²² 가

가 cytoskeletal actin actin microfilaments

collagenase 가 가

가 6,22-24 .

가 .

가 .

MMPs collagenase(MMP -1), gelatinase A(MMP -2),
matrilysin(MMP -7), gelatinase B(MMP -9) . 가

remodeling tissue -
derived inhibitors(TIMPs) . MMPs

elastin, fibronectin, proteoglycan core proteins 가

25,26 . MMPs

TIMPs , cytokines, tumor promoters, retinoids,
dexamethazone, colchicine 가

26 .

가 MMPs

가 27 .

MMPs TIMPs 가

가

MMPs TIMPs

MMP -2가 가 .

MMPs

TIMPs

가

가

V.

가

가

In vitro

1.

2. MTP

3. RT-PCR

1

mRNA

-actin

4. Zymogram

MMP -2

가

1

mRNA

MMP -2

가

TIMPs

가

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Abstract

**The effects of INF- γ and heparin on keloid fibroblast
collagen production**

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Many studies have examined the pathophysiology of keloids and hypertrophic scars but the exact cause is not known. Many different treatment methods have been introduced but none have shown satisfactory results.

It is known that heparin sulfate, an extracellular matrix component, is associated with cell adhesion, migration and proliferation in vivo and increases MMP activity while decreasing TIMP-1 activity. Also, heparin inhibits collagen synthesis of the keloid fibroblasts by down-regulating collagen gene expression. The reaction is prominent when ECGF is added. The immune response seems to be significant in the pathogenesis of keloids. Reductions in interferon- γ and tumor necrosis factor- α concentration clearly suggest that immune system, especially T-lymphocytes, plays an important role in the induction of collagen formation by fibroblasts. It is known that a reduced INF- γ is responsible for reduced collagenase activity. Furthermore, INF- γ inhibits the proliferation of fibroblasts.

To study the effects of heparin and INF- γ on keloid fibroblasts, we have

cultured the fibroblasts of keloid tissues from selected patients and investigated the amount of cell proliferation, collagen synthesis and expression of collagenase by MTT, RT-PCR and zymogram. The results showed that the study group exhibits a different amount of fibroblast proliferation and cell morphology compared to the control group. There was also a decrease in fibroblast activity in study group, when heparin or INF- γ was used, compared to the control group. The type I collagen mRNA expression was shown to decrease by heparin and INF- γ on quantitative RT-PCR results. MMP-2 activity was shown to increase in study group using zymogram.

As seen in the results, heparin and INF- γ inhibit keloid fibroblast activity, decrease synthesis of type I collagen mRNA and increases enzyme activity of MMP-2. This study may be a beginning of discovering new treatment methods in keloids and determining the pathophysiology of keloids.

Key Word : Keloid, fibroblast, interferon-gamma, heparin