

IGF가

Effects of insulin-like growth factor in peritoneal fluid of patients with endometriosis on the proliferation of endometrial stromal cells

Jung Gu Kim, Chang Seok Suh, Seok Hyun Kim,
Young Min CHoi, Shin Yong Moon and Jin Yong Lee

Department of Obstetrics & Gynecology, College of Medicine,
Seoul National University, Seoul 110-744, Korea

: IGF

** 1997 (: 04- 97- 021)

=Abstract=

The purposes of this study were to evaluate the effects of insulin-like growth factor(IGF)s in peritoneal fluid(PF) from patients with and without endometriosis on the proliferation of endometrial stromal cells and to investigate the effects of type I IGF receptor antibody on the response of endometrial stromal cells to PF from patients with endometriosis. IGFs in PF from patients with endometriosis(n=14) and without endometriosis(n=10) were measured by immunoradiometric assay and PF samples were divided into low IGF-I PF group(less than 85ng/ml) and high IGF-I PF group(more than 85ng/ml). Endometrial stromal cells from patients without endometriosis were cultured in serum free media in the presence or absence of 1% PF and thymidine incorporation test were used to evaluate the proliferation of endometrial stromal cells. Also cultures were incubated with type I IGF receptor monoclonal antibody (α IR3) before adding PF. PF from patients with endometriosis and without endometriosis increased thymidine incorporation in endometrial stromal cells. In patients with endometriosis, high IGF-I PF group had high IGF-II levels and resulted in higher thymidine incorporation than low IGF-I PF group but no significant difference in increase in thymidine incorporation between high IGF-I and low IGF-I PF group was noted in patients without endometriosis. There was not a significant correlation between increase in thymidine incorporation and IGF-I levels in PF from patients without endometriosis but in PF from patients with endometriosis. Preincubation with α IR₃ significantly inhibited the mitogenic response of endometrial stromal cells to PF. Our data indicate that IGF-I in PF may be involved in the growth of ectopic endometrium in patients with endometriosis.

Key Words: Endometriosis, IGF, Peritoneal fluid, Endometrial stromal cells,

가
10-15% .
(Ramey & Archer,
1993)

(Surrey & Halme, 1990)
가
가
(Deleon et al.,1986; Ylikorkala et al., 1986;
Mahmood & Templeton, 1991).

epidermal growth factor(EGF), fibroblastic growth factor(FGF)
가 (Huang,
et al.,1996).

(insulin-like growth factor; IGF) IGF-I
IGF-II 2 가 , (Jones & Clemmons,
1995). IGF transforming growth factor(TGF), EGF, FGF
(, 1996). IGF
(Halme & Hammond, 1995)

(Giudice et al.,1993; Zhou et al.,1994), (alveolar) (Rom et al.,1988),
(Arkins et al., 1993) IGF가
Giudice (1994) 7
IGF가 (1999)
IGF-I 가
IGF

IGF

가 I IGF αIR3
 가
 IGF가 가

(1)

, ,
 24 14 10
 . 가
 3
 1-2 .
 ()
 35 10
 . 가

(2)

. 1000 x g 10
 -70 가 .

(3)

IGFBP IGF

IGFBP-IGF IGF Mohan
 Baylink(1995) Bio-gel P 10 IGF .
 10g/L (bovine serum albumin; BSA), 0.1 M/L NaCl
 acetic acid(1M/L) 150 ml Bio-gel P-10 10g 16-18
 10-15 가 . 90-95% 가

(pellet) 0.2% collagenase 37 60 .
 DMEM/F-12/10% (fetal bovine serum;
 FBS) 2 38 μm (mesh)
 . 400 x g, 10
 37 , 5% CO₂ 30
 . (dominance)
 95% . 2 tryphan
 blue (cell viability test) 90% 가
 DMEM / F-12 / 10% FBS 5 x 10⁴/ml T-25
 5% CO₂ . 3-4 2 -196

6)

1-2 DMEM/F-12/10% FBS
 DMEM/MCDB(3:1)/1% FBS 96 24
 . 50 μg/ml ascorbic acid, 1mg/ml BSA, 5 μg/ml transferrin, herpes
 buffer, 1 μM/L progesterone DMEM/MCDB(3:1) ()
 (1%) 가 3
 . 가 가
 . 1 μCi ³H-thymidine 가 24 0.2% EDTA 50 μl
 (automatic cell harvester)
 . IGF
 가 가 1
 1 IGF αIR₃(1-15 μg/ml)
 thymidine incorporation .

7)

± .
 4 4
 . student's t-test
 paired t-test, p 0.05

1)

IGF

가

324.99 ± 16.50% thymidine incorporation

가

(p<0.0001).

322.06 ± 26.39%, 329.05 ± 16.09% thymidine incorporation

가 (p<0.0001).

IGF-I 가 85ng/ml

IGF-I ,

IGF-I

IGF-I

IGF-I

IGF-II 가

(p<0.0001) thymidine incorporation 가

(p<0.005).

IGF-I

IGF-I

IGF-II

thymidine incorporation 가

(Table 1).

thymidine

incorporation 가

IGF-I

가

(r =0.55,

p<0.01, Fig.1)

IGF-II

가

2) 1 IGF

가

1 IGF

가

thymidine incorporation

200.52 ± 17.36%

가 1 IGF

136.06 ± 16.41%

66.77 ± 14.31%

(p<0.05)(Fig. 2).

가 , (Celomic metaplasia theory), (Induction theory) 가 (Ramey & Archer, 1993) 가

IGF IGF - I 가 (mitosis) 가 IGF IGF - I IGF - II가 IGF (endocrine), (panacrine), (autocrine) (Jones & Clemmons,1995). IGF I II 가 IGF - I IGF - II 가 (membrane) tyrosine kinase I . Giudice (1994) 7 (1) (1999) IGF - I IGF IGF (Surrey & Halme, 1990)가 Koutsilieris (1991) Overton (1997) 가 가 가 가 IGF IGF - I IGF - I IGF - I IGF - I thymidine incorporation 가 IGF - I thymidine incorporation 가 Halme Hammond(1995) IGF - I

IGF-I 가

thymidine incorporation 가 IGF-I

가 . EGF, FGF

EGF FGF

(messenger ribonucleic acid) 가 (Hammond, 1993; Huang & Yeh,1994; Dunselman, 1995) . Huang (1996)

EGF bFGF 5 가

가

IGF IGF (IGF binding protein: IGFBP)

IGF IGFBP

(Jones & Clemmons, 1995). (1999) western ligand blot

IGFBP-3,

IGFBP-2, 26kDa IGFBP, 24kDa IGFBP IGFBP IGFBP-3

가

IGF-I

IGFBP-3

IGF IGF 가

1 IGF-I 가

IGF-I

IGF

가

IGF가

가

IGF

αIR3

가

1%

thymidine incorporation

IGF-I 가 85ng/ml (IGF-I) (IGF-I)

IGF-II 가 (p<0.0001) thymidine incorporation 가

(p<0.005).

incorporation

thymidine incorporation

IGF-I IGF-I IGF-II thymidine

IGF-I

IGF-I

Arkin S, Regeiz NM, Biragyn A, Reese DL, Kelley KW: Murine macrophages express abundant insulin-like growth factor-1 class I Ea and Eb transcripts. *Endocrinol* 1993, 133, 2334-2343.

DeLeon FD, Vijayakumar R, Brown M, Rao CV, Yussman MA, Schultz G: Peritoneal fluid volume, estrogen, progesterone, prostaglandin, and epidermal growth factor concentrations in patients with and without endometriosis. *Obstet Gynecol* 1986, 68,189-194.

Dunselman SAJ. Peritoneal environment in endometriosis. In: Shaw RW, eds,

Endometriosis ; current understanding and management. London: Blackwell Science Ltd., 1995, 47-74.

Giudice LC, Dsuspin BA, Jin IH, Vu TH, Hoffman AR: Differential expression of messenger ribonucleic acids encoding insulin-like growth factors and their receptors in human uterine endometrium and decidua. J Clin Endocrinol Metab 1993, 76, 1115-1122.

Giudice LC, Dsupin BA, Gargosky SE, Rosenfeld RG, Irwin JC: The insulin-like growth factor system in human peritoneal fluid ; Its effect on endometrial stromal cells and its potential relevance to endometriosis. J Clin Endocrinol Metab 1994, 79, 1284-1293.

Halme J. Hammond MG. The role of growth factors in endometriosis. In: Brosens I, Donnes J, eds. The current status of endometriosis. New York: The Parthenon Publishing Group, 1995, 211-219.

Hammond MG, Oh ST, Anners J, Surrey ES, Halme J: The effect of growth factors on the proliferation of human endometrial stromal cells in culture. Am J Obstet Gynecol 1993, 168, 1131-1138.

Huang JC, Papisakelariou C, Yusoff Dawood M: Epidermal growth factor and basic fibroblast growth factor in peritoneal fluid of women with endometriosis. Fertil Steril 1996, 65, 931-934.

Huang JC, Yeh J: Quantitative analysis of epidermal growth factor receptor gene expression in endometriosis. J Clin Endocrinol Metab 1994, 79, 1079-1101.

Jones JI, Clemmons DR: Insulin-like growth factors and their binding proteins : Biological actions. Endo Rev 1995, 16, 3-34.

, , , , , , :

1996, 39, 261-278.

, , , , , : IGF
IGFBP IGFBP-3 protease . 1999,
42,

Koutsilieris M, Michaud LA, Fortier M, Lemay A: Mitogens for endometrial-like cells can be detected in human peritoneal fluid. *Fertil Steril* 1991, 56, 888-893.

Mahmood TA, Templeton A: Peritoneal fluid volume and sex steroids in the preovulatory period in mild endometriosis. *Br J Obstet Gynaecol* 1991, 98, 179-183.

Mohan S, Baylink DJ: Development of a simple valid method for the complete removal of insulin-like growth factor(IGF)-binding proteins from IGFs in human serum and other biologic fluids ; Comparison with acid-ethanol treatment and C₁₈ Sep-pak separation. *J Clin Endocrinol Metab* 1995, 80, 637-647.

Overton CE, Fernandez-Shaew S, Hicks B, Barlow DH, Starkey P: In vitro culture of endometrial stromal and gland cells as a model for endometriosis: The effect of peritoneal fluid on proliferation. *Fertil Steril* 1997, 67, 51-56.

Ramey JW, Archer DF: Peritoneal fluid ; its relevance to the development of endometriosis. *Fertil Steril* 1993, 60, 1-14.

Rom WN, Bassett P, Fells GA, Nukiwa T, Trapnell BC, Crystal RG: Alveolar macrophages release an insulin-like growth factor-1 type molecule. *J Clin Invest* 1988, 82, 1685-1693.

Surrey ES, Halme J: Effect of peritoneal fluid from endometriosis patients on endometrial stromal cell proliferation in vitro. *Obstet Gynecol* 1990, 76, 792-797.

The American Fertility Society: Revised American Fertility Society classification of endometriosis. *Fertil Steril* 1985, 43, 351-352.

Zhou J, Dsupin BA, Giudice LC, Bondy CA: Insulin-like growth factor system gene

expression in human endometrium during the menstrual cycle. *J Clin Endocrinol Metab* 1994, 79, 1723-1734.

Ylikorkala O, Koskimies A, Laatkainen T, Tenhunen A, Viinikka L: Peritoneal fluid prostaglandins in endometriosis, tubal disorders and unexplained infertility. *Obstet Gynecol* 1984, 63, 616-620.