

IGF-1 Regulates Migration and Angiogenesis of Human Endothelial Cells

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Abstract. Recent studies revealed favorable para- and/or autocrine effects of IGF-1 in the pathogenesis of diabetic complications. On the other hand, hyperglycemia is a risk factor for the development of diabetic vascular complications. In this study we examined the effects of high glucose and/or IGF-1 on cell migration and angiogenesis (tubular formation) by using human endothelial cells (EC) *in vitro*. First we examined cell migration by the two-chamber method. Chronic treatment with a high concentration of D-glucose strongly stimulated the cell migration, which was mimicked by PMA, a protein kinase C (PKC) agonist. The cell migration was also induced by IGF-1. The glucose-induced cell migration was blocked by PKC inhibitor, H7. IGF-1-induced cell migration was not blocked by PD98059, MAPK/ERK kinase (MEK) inhibitor or wortmannin, a phosphatidylinositol (PI) 3-kinase inhibitor. Next we examined the effects of high glucose and/or IGF-1 on the tubular formation of EC. The tubular formation was induced only when the cells were exposed to a combination of high glucose and IGF-1. The tubular formation was blocked by MEK inhibitor and PI 3-kinase inhibitor but not by PKC inhibitor. These results indicate that hyperglycemia and IGF-1, respectively, stimulate the EC migration, and tubular formation is induced by a combination of IGF-1 and hyperglycemia.

Key words: Endothelial cells, Migration, Angiogenesis

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SEVERAL growth factors are regarded as risk factors for vascular diseases, such as atherosclerosis and diabetic microangiopathy, and angiogenesis is involved in the development of these diseases. There are many steps in angiogenesis: vascular endothelial cells (EC) release and migration from the basement membrane, and cell proliferation and tubular formation. We examined the effects of IGF-1 and glucose on several steps in the angiogenesis of human EC *in vitro*.

Materials and Methods

Cell line

ECV304 cells (Umbilical cord, endothelial, human) were cultured in RPMI 1640 supplement with 10% fetal bovine serum (FBS).

Cell migration assay

Cell migration assays were performed as previously described [1] with some modifications. F12K (serum free media) 0.7 ml, containing type 1 collagen (3 µg/ml), D-glucose and/or L-glucose (126–426 mg/dl), with or without IGF-1 (10⁻⁷ M), in the presence or absence of H7 [PKC inhibitor (1–100 µM)], PMA [PKC activator (100 nM)],

wortmannin [phosphatidylinositol (PI)-3 kinase inhibitor (100 nM)] or PD98059 [MAPK/ERK kinase (MEK) inhibitor (10 μ M)], were placed into a 24-well plate containing 8.0 μ m pore size cell culture inserts. Sub-confluent ECV304 cells were harvested with trypsin/EDTA, washed and resuspended to 10^6 cells/ml in F12K with 1%FBS, and 0.2 ml of the cells were placed into the inserts. The cells were incubated in CO₂ incubator at 37 °C and migration was measured after 4 h of incubation. All non-migrated cells were removed from the upper face of the membrane with a scraper, and migrated cells, those attached to the lower face, were fixed and stained with May-Gruenwald's and Giemsa's staining solution. Stained cells were counted under a microscope.

Tubular formation assay

Confluent ECV304 cells were washed with PBS and cultured with F12K serum free media with various concentration of IGF-1, D-glucose, with or without H7, LY294002 [PI-3 kinase inhibitor (10 μ M)] and PD98059 for 3 days. Then the EC were stained and the tubular formation was assayed under a microscope.

Calculation and Statistical Analysis

The statistical significance of differences in the results was evaluated by use of unpaired Student's t-test. A value of $P < 0.05$ was accepted as statistically significant.

Results

Cell migration

First we examined the effects of D-glucose and IGF-1 on EC migration. As shown in Fig. 1, a high concentration of D-glucose was a potent stimulator of EC migration, and also L-glucose stimulated EC migration by possibly its hyperosmolar effect, but the potency was less than that of D-glucose. The result suggest that D-glucose is metabolized and then protein kinase C (PKC) is activated in the cells. We therefore examined the effects of H7 (PKC inhibitor) and PMA (PKC agonist) on EC migration (Fig. 2). H7 suppressed EC migration in a dose dependent manner (data not shown), and PMA

enhanced cell migration. Thus, it was speculated that D-glucose stimulated EC migration through PKC activation. Next we examined the effect of IGF-1 on EC migration. As shown in Figs. 1 and 3, IGF-1 stimulated EC migration. Previous reports showed that several growth factors stimulate cell migration through PI-3 kinase [2], but in our study PI-3 kinase inhibitor (wortmannin) did not block the effect of IGF-1 on EC migration. On the other hand it is well known that growth factors activate MAPK pathway. The IGF-1-induced EC migration

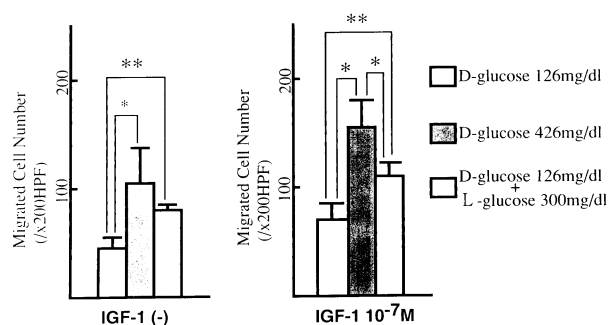


Fig. 1. The effect of D-glucose and L-glucose on EC migration. EC were incubated with D-glucose 126 mg/dl, D-glucose 426 mg/dl and D-glucose 126 mg/dl + L-glucose 300 mg/dl, respectively. After 4 h of incubation, EC migration was measured as METHODS. The values are shown as mean \pm SD. *: $P < 0.05$, **: $P < 0.01$.

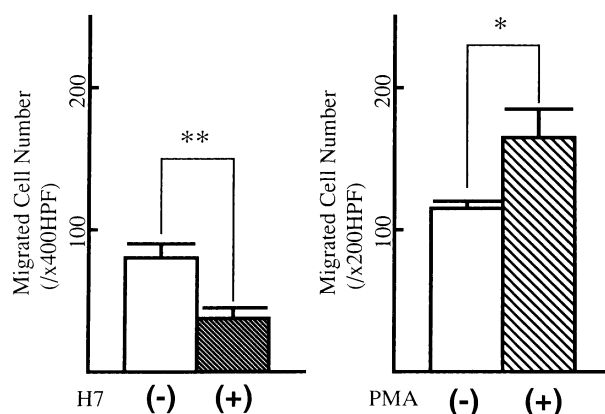


Fig. 2. The effect of H7 (PKC inhibitor) and PMA (PKC agonist) on EC migration. Left: EC were incubated with D-glucose 426 mg/dl in the presence or absence of H7 (10 μ M). Right: EC were incubated with D-glucose 126 mg/dl in the presence or absence of PMA (100 nM) for 4 h. Migrated EC were stained and counted as METHODS. The values are shown as mean \pm SD. *: $P < 0.05$, **: $P < 0.01$.

was not blocked by PD98059 (MEK inhibitor) treatment. The mechanism of EC migration induced by IGF-1 is unknown.

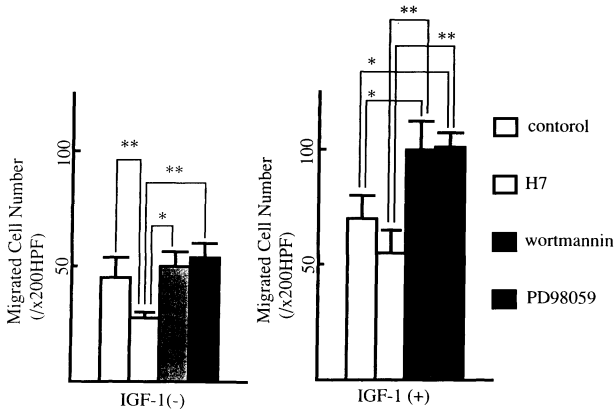


Fig. 3. The effect of IGF-1, wortmannin (PI-3 kinase inhibitor) and PD98059 (MEK inhibitor) on EC migration. EC were incubated with or without IGF-1 (10^{-7} M) for 4 h, and EC migration was measured. IGF-1 stimulated EC migration and that was not blocked by wortmannin (100 nM) and PD98059 (10 μ M). The values are shown as mean \pm SD. *: $P < 0.05$, **: $P < 0.01$.

Tubular formation

Confluent ECV304 cells were serum starved and then stimulated with various concentrations of IGF-1, D-glucose, with or without H7, wortmannin, LY294002 and PD98059 for 3 days. Fig. 4 shows that IGF-1 with high D-glucose promotes tubular formation, at the concentration of 10^{-8} – 10^{-6} M. IGF-1 or high D-glucose alone was not a strong promoter of angiogenesis. However, combination of IGF-1 and hyperglycemia potently stimulated tubular formation, and the effect was suppressed by PD98059 (Fig. 5). LY294002, but not H7 or wortmannin, also suppressed tubular formation of EC (data not shown). It was possible that tubular formation of EC induced by a combination therapy of IGF-1 and high D-glucose is through the activation of both PI-3 kinase and the MAPK pathways.

Discussion

Diabetic microvascular complications result from out of regulation of angiogenesis. In our study it

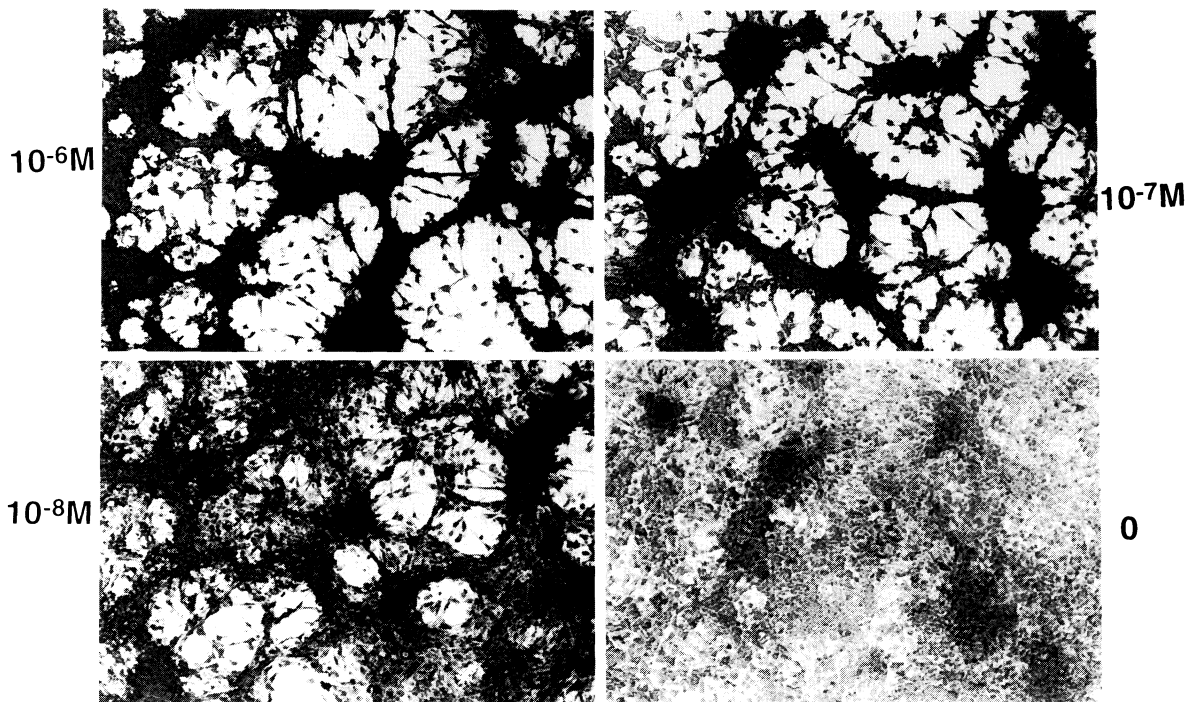


Fig. 4. The tubular formation of EC induced by high D-glucose (450 mg/dl) and IGF-1. The tubular formation was induced by IGF-1, at the concentration of 10^{-8} – 10^{-6} M.

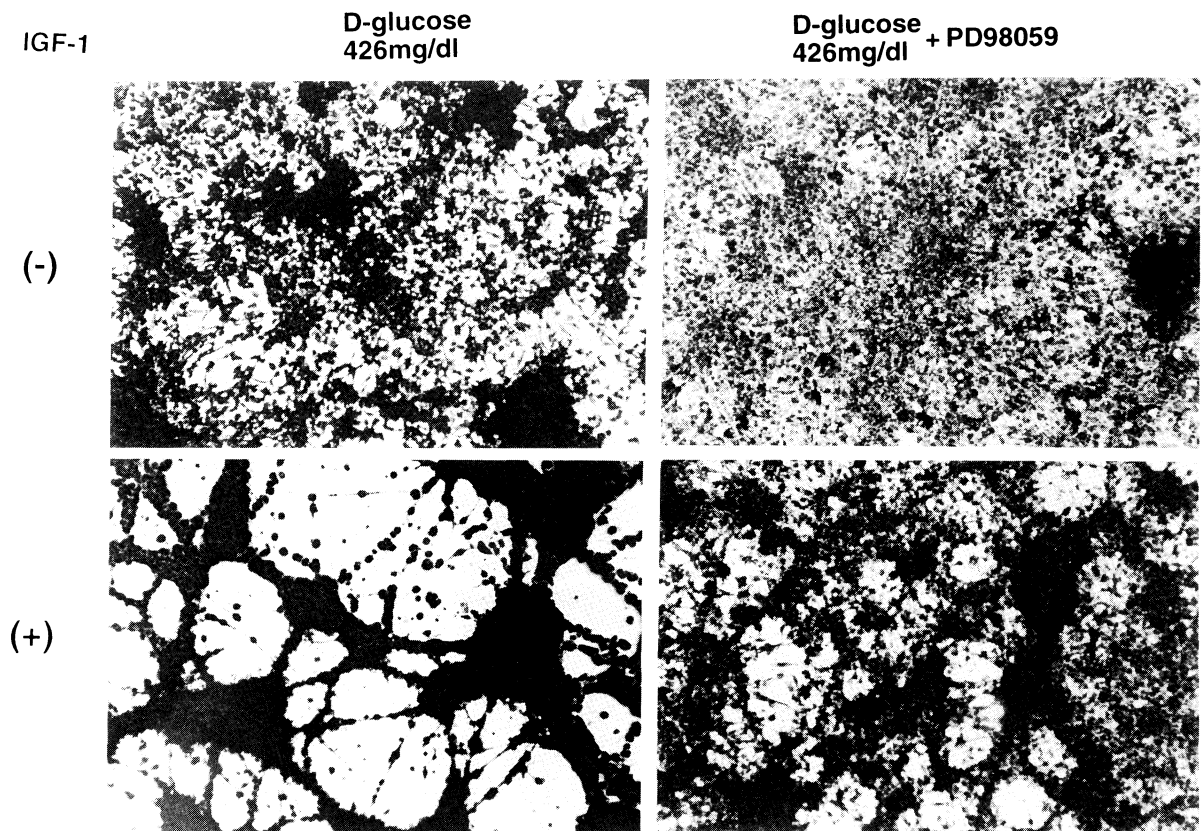


Fig. 5. The tubular formation of EC. The tubular formation was induced only when cells were exposed to a combination of high D-glucose and IGF-1. The tubular formation was suppressed by PD98059 (MEK inhibitor: 10 μ M).

was suspected that the activation of PKC by hyperglycemia and an unknown pathway of IGF-1, respectively, stimulate the endothelial cell migration. The tubular formation of EC was induced only by a combination of IGF-1 and

hyperglycemia, and this phenomenon was suppressed by PI-3 kinase inhibitor and MEK inhibitor. It was concluded that high glucose and/or IGF-1 regulate many steps in angiogenesis and are implicated in diabetic complications.

References

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