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Role of insulin-like growth factor-1 (IGF-1) in regulating cell cycle progression ☆

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ABSTRACT

Aims: Insulin-like growth factor-1 (IGF-1) is a polypeptide protein hormone, similar in molecular structure to insulin, which plays an important role in cell migration, cell cycle progression, cell survival and proliferation. In this study, we investigated the possible mechanisms of IGF-1 mediated cell cycle redistribution and apoptosis of vascular endothelial cells.

Method: Human umbilical vein endothelial cells (HUVECs) were pretreated with 0.1, 0.5, or 2.5 μg/mL of IGF-1 for 30 min before the addition of Ang II. Cell cycle redistribution and apoptosis were examined by flow cytometry. Expression of Ang II type 1 (AT_1) mRNA and cyclin E protein were determined by RT-PCR and Western blot, respectively.

Results: Ang II (1 μmol/L) induced HUVECs arrested at G_0/G_1, enhanced the expression level of AT_1 mRNA in a time-dependent manner, reduced the enzymatic activity of nitric oxide synthase (NOS) and nitric oxide (NO) content as well as the expression level of cyclin E protein. However, IGF-1 enhanced NOS activity, NO content, and the expression level of cyclin E protein, and reduced the expression level of AT_1 mRNA. L-NAME significantly counteracted these effects of IGF-1.

Conclusions: Our data suggests that IGF-1 can reverse vascular endothelial cells arrested at G_0/G_1 and apoptosis induced by Ang II, which might be mediated via a NOS–NO signaling pathway and is likely associated with the expression levels of AT1 mRNA and cyclin E proteins.

Introduction

Endothelial cells located in the vasculature serve as a barrier between the intravascular compartment and underlying tissues. They are usually exposed to various physical and biochemical stimuli, some of which may be detrimental to cell function. In order for endothelial cells to maintain functional integrity and hemostasis between the intravascular compartment and underlying tissues, mechanisms exist for purposes of adaptation or resistance to various stimuli. Regeneration of endothelium after vascular damage is an important factor that limits the development of atherogenesis [1]. Angiotensin II (Ang II) plays a central role in early atherogenesis and formation of atherosclerotic plaque. Previous studies showed that Ang II is a true cytokine at all stages of atherogenesis [2] and promotes the execution of programmed cell death [3].

Insulin-like growth factor-1 (IGF-1) is produced primarily by the liver as an endocrine hormone. The production of IGF-1 is stimulated by growth hormone and can be retarded by undernutrition, growth hormone insensitivity, and lack of growth hormone receptors among others. The primary action of IGF-1 is mediated by binding to specific IGF receptors present on many cell types in various tissues. IGF-1 is one of the most potent natural activators of the AKT signaling pathway, a stimulator of cell growth and multiplication and a potent inhibitor of programmed cell death. The over-expression of IGF-1 in myocytes protects them from apoptosis and interferes with myocyte hypertrophy by decreasing the expression levels of Ang II mRNA and AT1 mRNA in myocytes, further attenuating the response of myocytes to Ang II [4]. It is well known that inhibition of the renin–angiotensin system increases endothelial nitric oxide (NO) production [5]. Endothelial cells possess high affinity binding sites for IGF-1. The vasodilator effect of IGF-1 in the isolated perfused rat

Abbreviations: IGF-1, insulin-like growth factor-1; HUVECs, human umbilical vein endothelial cells; Ang II, angiotensin II; AT1, Ang II type 1; NOS, nitric oxide synthase; NO, nitric oxide; L-NAME, nomega-nitro-L-arginine methyl ester; PVDF, polyvinylidene difluoride; NO_x, NO metabolites; SD, standard deviation; VSMCs, vascular smooth muscle cells.

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kidney is abrogated by the NO synthase inhibitor nomega-nitro-L-arginine methyl ester (L-NAME). IGF-1 induces forearm vasodilation upon intra-arterial infusion into the brachial artery in healthy humans, which is completely reversed by addition of L-NAME [5]. However, whether IGF-1 plays a role in the cell cycle redistribution and apoptosis of vascular endothelial cells induced by Ang II is still unknown. In this study, we investigated the effects of IGF-1 on the enzymatic activity of NOS and resulting modifications in NO content, and the changes in expression levels of AT_1 mRNA and cyclin E protein in the vascular endothelial cells pretreated with 1 μmol/L Ang II.

Materials and methods

Cell culture and cell treatment. Human umbilical vein endothelial cells (HUVEC-12, ATCC, Manassas, VA, USA) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (DMEM, Hyclone, Logan, UT, USA), benzyl penicillin (l00 U/mL), and streptomycin (l00 μg/mL) in a humidified atmosphere containing 5% CO_2 at 37 °C. Cells (1×10^5/mL) were seeded in 6-well dishes and were starved for 24 h in DMEM with 1% FCS until the cells had reached subconfluence. Then cells were treated with 1 μmol/L Ang II (Sigma Chemical Co, St. Louis, MO, USA) for 24 h in the presence or absence of IGF-1 (Sigma Chemical Co, St. Louis, MO, USA).

To explore the effects of IGF-1 on cell cycle progression and apoptosis, human umbilical vein endothelial cells were pretreated with 0.1, 0.5, and 2.5 μg/mL of IGF-1 or 10^{-4} mol/L L-NAME (Sigma Chemical Co, St. Louis, MO, USA) for 30 min before addition of Ang II. Cell viability, NOS activity, NO content, AT_1 mRNA, and cyclin E protein were determined after treatment with 1 μg/mL Ang II for 24 h.

Analysis of cell cycle distribution and apoptosis. Cells were harvested and washed twice with phosphate-buffered saline followed by fixation in 80% ethanol for 30 min at room temperature. The cells were then collected by centrifugation and stained with 50 μg/μL propidium iodide. The cells were then treated with 100 μg/μL RNase for 15 min at 37 °C followed by analysis using a FACScan flow cytometer (American Coulter EPICS XL flow cytometer, system II software). The fluorescence intensity of 1×10^5 cells for each sample was quantified.

Semi-quantitative RT-PCR analysis of AT_1 mRNA. Total RNA in endothelial cells (8×10^6 cells) was isolated using the TRIZOL reagent. Reverse transcription-polymerase chain reaction (RT-PCR) was performed using a TaKaRa one step RT-PCR kit based on the manufacturer's instructions. A total of 1 μg of total RNA served as a template for each reaction. For amplification, a primer pair for human AT_1 was as follows: sense primer, 5′-ATGCCATCCCA GAAAGTCG-3′, antisense primer, 5′-ATTCCCACCAC AAAGATGA TACTG-3′. Reverse transcription was performed at 50 °C for 15 min. For PCR, 35 cycles were used at 94 °C for 2 min, 94 °C for 30 s, 56 °C for 36 s and 72 °C for 40 s. β-Actin was amplified as a reference for quantification of AT_1 mRNA. Densitometric scanning to quantify amounts of RT-PCR product was performed using an Eagle Eye II Imageware system. The signal intensity of each AT_1 band was normalized to that of β-actin.

Western blot analysis of cyclin E protein. Sample preparation and Western blot analyses were performed as described below. Briefly, cell lysates were separated on 8% SDS–PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane followed by a 12 h incubation in blocking solution at 4 °C (Tris-buffered saline containing 5% nonfat dried milk and 0.05% Tween 20). Rabbit anti-human cyclin E antibody (BD Pharmingen, USA) at a dilution of 1:500 was reacted with the blots overnight at 4 °C. After washing, the blots were incubated with goat anti-rabbit IgG1 horseradish peroxidase-conjugated antibody (BD Pharmingen, USA) as the secondary antibody at 1:1000 dilution for 2 h at room temperature. The membranes were visualized using the ECL kit (enhanced chemiluminescence, Santa Cruz). Densitometric measurements were performed using an Eagle Eye II Imageware system. β-Actin was used as the internal control.

Determinations of NO content and NOS activity. The amount of NO released in HUVECs was assessed by evaluating the concentrations of NO metabolites (NO*x*), i.e., nitrite plus nitrate. Briefly, NO*x* concentrations were evaluated by colorimetric detection of nitrite after conversion of sample nitrate to nitrite. NOS activity was also measured by colorimetric detection according to manufacturer's instructions.

Statistical analysis. All data were expressed as mean ± standard deviation (SD). Data analyses were done with SPSS software (Version 13.0; SPSS, Chicago, IL). Data among different groups were compared using one-way ANOVA or a Newman–Keuls–Student test. *P* values less than 0.05 was considered statistically significant.

Results

Effect of IGF-1 on cell cycles and apoptosis

As shown in Tables 1 and 2, it was determined that the IGF-1 + Ang II treatment group significantly reduced the apoptotic ($P < 0.01$) and G_0/G_1 phase ($P < 0.01$) cells, enhanced the cell numbers at the S phase ($P < 0.01$) and G_2/M phase ($P < 0.01$) compared with the Ang II treatment group, and reached the maximal effects when HUVECs were incubated for 24 h. However, after the HUVECs were pretreated with 100 μmol/L L-NAME for 30 min, our data showed that the Ang II + IGF-1 + L-NAME treatment group significantly enhanced the apoptotic ($P < 0.05$) and G_0/G_1 phase cells ($P < 0.05$), and reduced the cell numbers at the S phase ($P < 0.05$) and G_2/M phase ($P < 0.05$) compared with the IGF-1 + Ang II treatment group (Fig. 1).

Effect of IGF-1 on the expression of AT_1 mRNA

To further understand the role of IGF-1 on endothelial cell cycle and apoptosis regulation, we investigated the effect of IGF-1 (0.5 μg/mL) on the expression of AT_1 mRNA in HUVECs.

Table 1
Effects of IGF-1 and L-NAME on cell cycle progression and apoptosis of endothelial cells.

Group	Apoptosis (%)	G_1/G_0 (%)	S (%)	G_2/M (%)
Control	0.96 ± 0.29	74.7 ± 0.28	18.07 ± 0.09	7.20 ± 0.17
Ang II	2.55 ± 0.042*	88.13 ± 0.38*	7.43 ± 0.15*	4.43 ± 0.24*
Ang II + IGF-1	0.28 ± 0.015##	48.27 ± 0.49##	38.07 ± 0.23##	13.67 ± 0.26##
Ang II + IGF-1+L-NAME	1.21 ± 0.015+	65.57 ± 0.49+	27.30 ± 0.35+	7.10 ± 0.23+

Data are presented as mean ± SD ($n = 6$).
# $P < 0.05$.
$P < 0.01$ compared with Ang.
* $P < 0.05$ compared with control.
+ $P < 0.05$ compared with Ang II + IGF-1.