Effects of High Glucose on Interleukin-6 Production in Human Mesangial Cells

Interleukin (IL)-6 is an autocrine growth factor for mesangial cells. It is not known whether high glucose influences IL-6 production in mesangial cells. Angiotensin II (AGII) is involved in the progression of renal diseases including diabetic nephropathy. Therefore, we evaluated the effects of high glucose in concert with AGII on IL-6 production in human mesangial cells and the modulation by blocking AGII. After 48 hr of culture, IL-6 mRNA expression was analyzed by reverse transcription and polymerase chain reaction (PCR). Quantitative determination of IL-6 concentrations in the culture supernatants of mesangial cells was performed using a sandwich enzyme immunoassay kit. Incubation of mesangial cells with high glucose (450 mg/dL) reduced the ratio of PCR products for IL-6 to β -actin on densitometric results, while AGII (10^{-7} M) increased it. The IL-6 secretion in the supernatant was also increased by AGII and decreased by high glucose. The IL-6 mRNA expression and IL-6 secretion in combination of high glucose and AGII were higher than those in high glucose and similar with those in control media. The addition of losartan (10^{-6} M) or captopril (10^{-6} M) to high glucose had no additional effects on IL-6 production. These results suggest that whereas AGII increases IL-6 production, high glucose decreases it. The IL-6 production of mesangial cells in diabetic milieu may be complicated and depend on the local effects of high glucose and/or AGII.

Key Words : Glucose; Angiotensin II; Interleukin-6

Chun-Gyoo Ihm, Jae Kyung Park,
Hee Jin Kim, Tae Won Lee,
Dae Ryong Cha*

Department of Internal Medicine, Kyung Hee
University, Seoul; Department of Internal Medicine*,
Korea University, Seoul, Korea

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Address for correspondence

Chun-Gyoo Ihm, M.D.
Department of Internal Medicine, Kyung Hee
University Medical Center, 1 Hoiki-dong,
Dongdaemun-gu, Seoul 130-702, Korea
Tel : +82-2-958-8188, Fax : +82-2-968-1848
E-mail : CGIhm@chollian.net

INTRODUCTION

Interleukin (IL)-6 is an autocrine growth factor for mesangial cells (1). IL-6 stimulates the proliferation of mesangial cells and also enhances fibronectin expression by human mesangial cells (2). It was reported that in situ IL-6 mRNA expression was associated with mesangial cell proliferation in the patients with diabetic nephropathy (3). However, the mesangial cell proliferation is not a typical finding of diabetic nephropathy, but acellular area can be shown in the patients with nodular diabetic glomerulosclerosis. It has been reported that high glucose decreased the cellularity of mesangial cells (4, 5). It is not, however, reported whether high glucose influences IL-6 production in mesangial cells. The results that observed in the peripheral blood mononuclear cells were controversial (6, 7).

There is evidence that the intrarenal renin-angiotensin system is activated in diabetic nephropathy (8). Angiotensin II (AGII) is involved in the progression of renal diseases including diabetic nephropathy (9). AGII induces the synthesis of matrix proteins and several cytokines and growth factors including IL-6 in mesangial cells (10-13). The effects of AGII

blocking agents on IL-6 expression in mesangial cells are not yet clearly elucidated (13).

Therefore, this study was designed to evaluate the direct effects of high glucose in concert with AGII on IL-6 mRNA expression or IL-6 secretion in mesangial cells and the modulation by blocking AGII with losartan, an AGII receptor blocker, or captopril, an ACE inhibitor.

MATERIALS AND METHODS

Human mesangial cell culture

Portions of normal renal cortex were obtained from human kidneys immediately after surgical nephrectomy. Collagenase (Gibco BRL, Gaithersburg, MD, U.S.A.) treated glomeruli were plated on culture dishes in DMEM medium (Gibco BRL) containing 17% heat-inactivated fetal bovine serum (Sigma Chemical Co., St. Louis, MO, U.S.A.) (14). Cells displayed the typical spindle or stellate morphology. Immunofluorescent staining of the cells with antibodies to common leukocyte antigen and factor VIII was negative, and the cells were

capable of growth in D-valine substituted medium. Near confluent mesangial cells in the third to fifth passage were transferred to a 24-well plate, and were rested in serum-free medium for 72 hr. Then mesangial cells were cultured in the media containing 0.5% heat-inactivated fetal bovine serum with D-glucose (Sigma, Louis, U.S.A.), AGII (Sigma, Louis, U.S.A.), captopril (Sigma, Louis, U.S.A.), or losartan (Merck, Whitehouse Station, U.S.A.). The concentrations of AGII (10^{-7} M), captopril (10^{-6} M), or losartan (10^{-6} M) were used as previously described (15, 16). Each group had three to four separate experiments.

Reverse transcription (RT)-polymerase chain reaction (PCR)

Mesangial cells were resuspended in RNazol (Cinna/Bio-tecx, Houston, TX, U.S.A.) and RT was performed using RT kit (Boehringer, Mannheim, Melbourne, Australia), as previously described (17). The reaction mixture was incubated for 60 min at 42°C and heated for 7 min at 90°C in thermocycler (Crocodyle III, Oncor Inc., Gaithersburg, MD, U.S.A.). In order to quantitate the PCR products comparatively and confirm the integrity of the products, we coamplified β -actin in a companion tube. The primers based on the known sequences of human cDNAs were as follows. β -actin: sense was 5' CCC CAG GCA CCA GGG CGT GAT 3' and antisense 5' GGT CAT CTT CTC GCG GTT GGC CTT GGG GT 3'. IL-6: sense was 5' GCG CCT TCG GTC CAG TTG 3' and antisense 5' CTC CTT TCT CAG GGC TGA G 3'. RT products were amplified with sense and anti-sense primers in a reaction mixture: 10 mM Tris HCl (pH 8.3), 200 μ M dNTP, 1.25 U *Taq* DNA polymerase, 1.5 mM $MgCl_2$, 40 pmol for IL-6 and 20 pmol for β -actin primer. The mixture was heated for 3 min at 94°C, and the PCR cycle consisted of 1 min denaturation at 94°C, 1 min annealing at 60°C for β -actin and 55°C for IL-6, 3 min extension at 72°C and final extension for 7 min at 72°C. After amplification, PCR products were electrophoresed through a 1% agarose gel with 0.5 μ g/mL ethidium bromide to visualize the DNA bands. The gel was photographed with Polaroid Type 665 positive/negative film (Polaroid Corporation, Cambridge, Massachusetts, U.S.A.) (18). The bands on the negative film were scanned by densitometry (Computer assisted dual wave length flying spot scanner, CS-9000, Shimadzu Co., Kyoto, Japan). The ratio of PCR products for IL-6 to β -actin according to densitometric results also showed maximal sensitivity at 2 μ L of cDNA and 38 cycles of PCR that we used in this study.

Sandwich enzyme immunoassay

Quantitative determination of IL-6 concentrations in the culture supernatants of mesangial cells was performed using a sandwich enzyme immunoassay kit (Quantikine; R&D Inc., Minneapolis, MN, U.S.A.). Standards and samples were pipet-

ted into the wells. After washing away unbound substances, an enzyme-linked polyclonal antibody was added to the wells. Following a wash, a substrate solution was added to the wells and the intensity of the color was measured.

Statistics

Results are expressed as the mean $\pm$ SD. Comparisons among groups were made by analysis of variance, and comparisons between groups were made using the Student's unpaired t-test or the Mann-Whitney test. Significance was assigned at the $p < 0.05$ levels.

RESULTS

Effects of high glucose or AGII on IL-6 mRNA expression

PCR products, 211 bp in length corresponding to IL-6 mRNA and 263 bp to β -actin mRNA, were detected from mesangial cells. We evaluated the effects of high glucose and AGII on IL-6 expression in mesangial cells. After 48 hr of culture, IL-6 mRNA expression was measured by RT-PCR. Incubation with AGII (10^{-7} M) enhanced IL-6 expression by 30%, but in contrast, high glucose (450 mg/dL) reduced IL-6 expression by 33% (Media 0.86 ± 0.14 , AGII 1.12 ± 0.05 , high glucose 0.58 ± 0.15 ; $p < 0.05$) (Fig. 1, 2). The IL-6 mRNA expression (0.95 ± 0.25) in combination of high glucose and AGII was similar to those in media (0.86 ± 0.14) and higher than those in high glucose (0.58 ± 0.15).

Fig. 1. Representative agarose gel analyzes of RT-PCR assays performed to detect IL-6 message in human mesangial cells incubated with media alone, angiotensin II (AGII) (10^{-7} M), high glucose (HG; 450 mg/dL), or high glucose with angiotensin II, captopril (C; 10^{-6} M) or losartan (L; 10^{-6} M).

Fig. 2. Effects of angiotensin II (AGII), high glucose (HG), or high glucose with angiotensin II, captopril (C) or losartan (L) on IL-6 mRNA expression in human mesangial cells. * $p < 0.05$ vs. media alone; † $p < 0.05$ vs. HG.

Effects of high glucose or AGII on IL-6 secretion

Similar to the results of IL-6 mRNA expression, the IL-6 secretion in the supernatant was increased by AGII and decreased by high glucose (Media 18.0 ± 3.2 , AGII 28.0 ± 4.0 , high glucose 9.9 ± 1.0 pg/mL) (Fig. 3). The IL-6 secretion (20.0 ± 4.0 pg/mL) in combination of high glucose and AGII was also similar to those in media (18.0 ± 3.2 pg/mL) and higher than those in high glucose (9.9 ± 1.0 pg/mL).

Effects of losartan or captopril

Furthermore, we tested the effects of losartan (10^{-6} M) or captopril (10^{-6} M) administration on IL-6 production by mesangial cells in the presence of high glucose and fetal bovine serum. The administration of these drugs at these concentrations to high glucose had no additional effects on the IL-6 mRNA expression (high glucose 0.58 ± 0.15 , high glucose+captopril 0.63 ± 0.25 , high glucose+losartan 0.68 ± 0.20 ; $p > 0.05$) and secretion (high glucose 9.9 ± 1.0 pg/mL, high glucose+captopril 10.7 ± 2.0 pg/mL, high glucose+losartan 12.2 ± 5.0 pg/mL; $p > 0.05$) (Fig. 1-3).

DISCUSSION

Various factors such as IL-1, tumor necrosis factor α , lipopolysaccharide, or immune complex stimulate IL-6 production (2, 12, 19), but the effect of high glucose on IL-6 expression in human mesangial cells has not been reported yet. In peripheral mononuclear cells there are controversies about the effect of high glucose on IL-6 production. The results of one study demonstrated that IL-6 production was suppressed by

Fig. 3. Effects of angiotensin II (AGII), high glucose (HG), or high glucose with angiotensin II, captopril (C) or losartan (L) on IL-6 secretion in human mesangial cells. * $p < 0.05$ vs. media alone; † $p < 0.05$ vs. HG.

elevated glucose concentration dose- and time-dependently (6). Elevated glucose levels significantly alter the cytokine production and concomitantly inhibit cellular proliferation. In contrast, other study showed that hyperglycemia or advanced glycosylation end products stimulated IL-6 synthesis and secretion (7). IL-6 levels of culture supernatants incubated with 22 mM or 33 mM glucose showed considerable increase over basal levels incubated with 11 mM glucose, whereas those levels incubated with high concentration of mannitol showed no increase.

In our study, high glucose reduces IL-6 mRNA expression and IL-6 secretion in human mesangial cells. It was reported that high glucose increases the production of transforming growth factor (TGF) β and decreases the cellularity of mesangial cells (4, 5). High glucose-induced decrease of IL-6 may also be related to the decrease of cellularity in mesangial cells when incubated in high glucose. The results in other cells indicated that TGF β 1 production is preceding the reduced IL-6 production in condition of high glucose concentration (6). Therefore, the decrease of IL-6 production in concert with increase of TGF β 1 may be involved in the early development of diabetic glomerular hypertrophy. This may be different from the data in the late stage of diabetic nephropathy that in situ IL-6 mRNA expression was associated with mesangial cell proliferation in the patients with diabetic nephropathy (3). It is, however, unclear how high glucose reduced IL-6 expression. Further studies are necessary to investigate the underlying mechanisms including intracellular transduction pathway.

There is evidence that the intrarenal renin-angiotensin system is activated in diabetic nephropathy (8) and that signals for renin, angiotensinogen and ACE mRNA are stronger in mesangial cells of patients with diabetes mellitus (20). It has

been reported that AGII receptors are present in abundance in mesangial cells (21). Our study showed that AGII induced the increase in IL-6 mRNA expression or IL-6 secretion. Other study showed that AGII enhances DNA synthesis of mouse mesangial cells through the mediation of IL-6 (10). AGII has been also observed to induce cell hypertrophy (11).

It has been reported that hyperglycemia enhances the expression of AGII receptors and the local effects of AGII (22). These suggest that hyperglycemia can act in synergy with locally increased AGII levels in inducing diabetic glomerular hypertrophy (23). Therefore, the combined administration of high glucose and AGII may be useful to understand the mechanisms in the development of diabetic nephropathy. Our study showed that the IL-6 mRNA expression or IL-6 secretion in combination of high glucose and AGII were higher than those in high glucose alone and similar to those in media. These indicate that the local effect of AGII may be antagonistic to that of high glucose on IL-6 expression in diabetes. In situ hybridization study showed that IL-6 mRNA is expressed by glomerular resident cells and interstitial cells in the renal tissue of patients with diabetic nephropathy and that its expression may be associated with mesangial proliferation (3). The IL-6 production of mesangial cells in diabetic milieu may thus be complicated and depend on the local effects of high glucose and/or AGII. We can speculate that localized IL-6 production in mesangial cells may be decreased at the stage of early diabetes and increased, at least partly by AGII, at the late stage of diabetic nephropathy. At the late stage the combined effects of AGII and IL-6 may aggravate mesangial injury.

Our study showed that the administration of losartan or captopril to mesangial cells cultured with high glucose and fetal bovine serum had no additional effects on IL-6 mRNA expression and IL-6 secretion. These indicate that AGII may be not directly involved in the high glucose-induced alterations in IL-6 production. In other study administration of valsartan in therapeutic dosages in normal glucose concentrations did not influence stimulated production of IL-6 by whole blood (13). It's unclear whether these findings may reflect in vivo state and whether other concentrations of these drugs may have some effects.

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