Original Article
Advanced glycation end-products affect the permeability of rat glomerular endothelial cells via Rac-1 signaling pathways

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Abstract: Aim: The current study aimed to determine the effects of advanced glycation end products (AGEs) on rat glomerular endothelial cell (GECs) permeability and to explain whether the mechanisms of AGEs-mediated changes of rat GECs permeability are associated with Rac1 signal pathways. Methods: In vitro cultured primary rat GECs, with different concentrations of AGEs-modified human serum albumin (AGEs-HSA) stimulating rat GECs with different times, established the control group for comparison. Transendothelial electrical resistance of transendothelial resistance (TER) and fluorescein-isothiocyanate bovine serum albumin (FITC-BSA) average permeability coefficients were used to detect the permeability of GECs monolayer cells. At the same time, this study detected changes in Rac1 activity using pull-down assay technology. Results: 1) Rat primary GECs were isolated and cultured in vitro. They were identified by an inverted microscope; 2) AGEs stimulation was applied after incubation for 8 hours. With an increase of AGEs concentrations, the mean value of TER of GECs cell monolayers gradually decreased, but the average permeability coefficient of FITC-BSA gradually increased. Results suggest that the permeability of GECs cell monolayers increased as concentrations of AGEs increased, showing a dose-dependent relationship. Concentrations of 100 μg/mL AGEs stimulated the GECs cell monolayers, after incubation. With stimulation times of AGEs prolonged, the mean value of TER of GECs cell monolayers decreased gradually and the average permeability coefficient of FITC-BSA increased, suggesting that permeability of GECs cell monolayers increased gradually with stimulation times, indicating time dependence. This effect was inhibited by 2'-O-methyladenosine-3',5'-cyclic monophosphate (O-Me-cAMP); 3) The level of Rac1 activity, but not total Rac1 level, in the AGE-HSA (100 μg/mL) treatment group was significantly decreased, compared to the control group. However, HSA did not exhibit this effect. Results suggest that Rac1 signaling pathway plays an important role in mediating the function of AGE-induced functional changes in endothelial cells. Conclusion: The permeability of rat GECs cell monolayers increases with concentrations of AGEs, showing dose-dependence and time-dependence. Rac1 signaling pathways play an important role in the morphological and functional changes of GECs mediated by AGEs.

Keywords: Glomerular endothelial cells, transendothelial resistance, permeability, Rac1, advanced glycation end products

Introduction

Diabetes and its complications seriously harm human health. Diabetes has become a very difficult problem to address in public health, worldwide. Nearly 90 million adults in China have diabetes and nearly 150 million adults are in pre-diabetes [1]. About 20-30% of diabetic patients develop diabetic nephropathy (DN). Some of them even develop end-stage renal disease (ESRD) [2-4], which brings a heavy medical burden to patient families and society in general [5-7]. The pathogenesis of diabetic nephropathy is complex. It has not been fully elucidated, at present, but its basic pathological changes are renal microangiopathy, which is clinically manifested as proteinuria. Continuous proteinuria accelerates the progression of diabetic nephropathy [8, 9]. An important landmark event of diabetic patients complicated...
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with diabetic nephropathy is the increase of renal glomerular microvascular permeability [10, 11], namely the increase of glomerular capillary permeability and the generation of proteinuria. Recent studies have suggested that an increase of GECs permeability plays a key role in the production of proteinuria in diabetic patients [12-14].

In recent years, studies have shown that, in diabetic patients, many advanced glycosylated end products (AGEs) form. Long-term sustained hyperglycemia plays a key role in the progression of diabetic nephropathy [15, 16]. Recent studies have found that AGEs can cause GECs damage, mainly manifesting as changes in GECs morphology and function. This leads to leakage of macromolecular substances, such as albumin, out of vessels [17, 18]. Some researchers have also found that AGEs induce increased permeability of skin microvascular endothelial cells in a time-dependent and dose-dependent manner, according to in vitro cell culture experiments [19]. Based on the above, it is reasonable to speculate that AGEs can lead to increased permeability of GECs in diabetic patients. However, there are still few studies concerning the increased permeability of GECs in diabetic patients caused by AGEs, as well as its mechanisms.

In this study, isolated cultured normal rat GECs were planted on Transwell chambers to construct in vitro monolayer cells. Normal GECs monolayers were stimulated by AGEs. Transendothelial resistance (TER) of monolayer cells and permeability of fluorescein-isothiocyanate bovine serum albumin (FITC-BSA) were measured, respectively, to clarify the influence of AGEs on the permeability of GECs monolayers in rats. In addition, levels of Rac1 activity were investigated using a pull-down assay. Potential involvement of Rac1 signaling pathways in this process was examined. The present study aimed to improve the understanding of the pathogenesis of nephropathic proteinuria in diabetics, providing novel therapeutic targets for diabetic nephropathy.

Materials and methods

Materials

Dulbecco’s Modified Eagle’s medium, MCDB-131, trypsin, and fetal bovine serum (FBS) were purchased from Gibco Life Technologies (Carlsbad, CA, USA). Vascular endothelial growth factor was purchased from BD Biosciences (San Jose, CA, USA) and fluorescein isothiocyanate (FITC)-phalloidin was obtained from Molecular Probes Life Technologies (Grand Island, NY, USA). FITC-labeled goat anti-rabbit antibody, human serum albumin (HSA), and FITC-bovine serum albumin were obtained from Sigma-Aldrich (St. Louis, MO, USA). DAPI was purchased from Invitrogen Life Technologies (Carlsbad, CA, USA) and 2'-O-methyladenosine-3',5'-cyclic monophosphate (O-Me-cAMP) was purchased from Aladdin Reagents Co., Ltd (Shanghai, China). Transwell chambers were purchased from Corning Life Sciences (Tewksbury, MA, USA) and a Rac1 Activity Detection kit was purchased from EMD Millipore (Billerica, MA, USA). A Bradford kit was purchased from Shenneng Bocai Biotechnology Co., Ltd. (Shanghai, China).

Isolation and culture of GECs

Isolation and culturing of primary GECs was conducted, as previously described [20, 21]. All studies were approved by the Ethics Committee of Anhui Provincial Hospital (Hefei, China) for Animal Experiments and conformed to the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health. Primary GECs were isolated from the kidneys of two male Wistar rats (body weight, 80-120 g) (Animal Department of Anhui Medical University, China; certificate no. 003). Primary GECs were cultured in MCDB131 medium supplemented with 10% FBS in a humidified atmosphere with 5% CO2. All operations were performed under 10% chloral hydrate (Hechang Chemical Company, Wuhan, China) and all effort was made to minimize the suffering of the animals.

Preparation of AGE-modified HSA

AGE-HSA was prepared by incubating HSA with glucose, as previously described [22-25]. The reaction system contained 1.5 g HSA and 3.0 g D-glucose (Meilun Bio, Dalian, China), which were dissolved in 100 mL phosphate-buffered saline (PBS; 0.2 mol; pH 7.4; Gibco Life Technologies) and filtered with 0.22 μm microporous membranes (EMD Millipore). The solution was then maintained in a container filled with nitrogen. It was sealed, protected from light, and incubated at 37°C for three months. Unbound materials were removed using a dialysis bag (molecular weight, 10,000; Corning,
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Inc., Corning, NY, USA). The same procedure was completed without the addition of D-glucose for controls. AGE values of the samples (1 mg/mL) were detected by fluorescence scanning (BX43; Olympus Corp., Tokyo, Japan) and samples were stored at -20°C.

Detection of transcellular resistance of GECs in rats

According to reported methods [26, 27], two stx-2 electrodes were placed in the upper and lower Transwell chambers, respectively, according to design requirements of the experiment. They were then connected with the cross-cell resistance voltage ohmmeter to measure the blank electrical impedance of Transwell's small chamber for 3 consecutive measurements, calculating the average value. After GECs monolayers were constructed, the same method was used to detect monolayer electrical impedance of GECs in Transwell rats at a specific time. The blank electrical impedance of each chamber was subtracted, then multiplied with the bottom area of the Transwell chamber. TER values were then obtained.

Cell permeability analysis

Using a previously reported method [28], GECs were seeded into the top compartment of a Transwell chamber with FITC-albumin (100 μl; 1 mg/mL; Sigma-Aldrich). Subsequent to incubation, fluorescence intensity of samples was analyzed using an HTS-7000 Bio Assay Reader (BioAssay Systems, Hayward, CA, USA) with 495 nm excitation and 520 nm emission filters. The apparent permeability coefficient \( (P_a) = (F/t) (1/A) (v/L) \) was used, where \( F \) indicates the fluorescence intensity in the bottom chamber, \( t \) indicates time (sec), \( A \) is the membrane area (cm\(^2\)), \( v \) is the solution volume in the bottom chamber, and \( L \) indicates the fluorescence intensity in the top chamber. Results are expressed as a percentage \( (P_a\% = (\text{experimental } P_a \text{ value/control } P_a \text{ value}) \times 100\%) \). These experiments were repeated a minimum of five times.

Rac1 activity analysis

Rac1 activity was analyzed using a pull-down assay, as previously described [29]. Protein was extracted using a chemical cleavage method and protein concentrations were detected using the Bradford assay. Samples (50 μg) underwent SDS-PAGE (10%) and were subsequently transferred to polyvinylidene difluoride membranes (EMD Millipore). The membranes were incubated with RAC1-GTP antibody and were incubated with horseradish peroxidase-conjugated secondary antibodies (Zhongshan Jingqiao Company, Beijing, China). Chemiluminescent images were obtained using a Kodak Image Station 2000R system (Kodak, Rochester, NY, USA). Results were analyzed using Image J software version 1.44e (National Institutes of Health, Bethesda, MD, USA).

Rac1 protein was determined by western blot

The abovementioned collected protein samples (20 μl) were loaded onto the SDS-PAGE and electro blotted to nitrocellulose membranes. Membranes were incubated with primary antibody CAV-1 (British Abcam company) (1:1000) overnight at 4°C after being blocked with 5% nonfat milk for 12 hours. Protein bands, combined with anti-rabbit secondary antibody (Beijing yanji biological reagent co, LTD) (1:25000), were observed using the enhanced chemiluminescence (ECL) detection system and quantitated with optical density values.

Statistical analysis

Statistical analysis was performed using SPSS software, version 13.0 (SPSS, Inc., Chicago, IL, USA). Data are presented as mean ± deviation. One-way ANOVA was used to compare average TER and FITC-BSA permeability coefficients of GECs monolayers between the blank control group and observation group. \( P < 0.05 \) indicates statistically significant differences.

Results

GECs were successfully isolated and cultured

After successful isolation of GECs in the rat primary generation, cells were cultured for 3 days and observed under an inverted microscope for elliptic or long fusility of the primary generation. After 7 days of culturing, cells were grown and fused into a single layer. Cells presented an inlaid arrangement, presenting a pavers-like structure with contact inhibition (Figure 1). Identification of isoplant hemagglutinin binding tests using fluorescence isothiocyanate: GECs and isothiocyanate fluorescein binding tests
were positive, showing bright yellowish green fluorescence around the cells (Figure 2).

**Influence of AGEs-HSA on GECs permeability in rats: different concentrations of AGEs-HSA on permeability of GECs monolayer after stimulation for the same time**

After the third generation of GECs in rats were inoculated with Transwell to construct the monolayers, different concentrations (25 μg/mL, 50 μg/mL, and 100 μg/mL) of AGEs-HSA were selected to incubate GECs monolayer for 8 hours. Effects of AGEs on GECs permeability were evaluated in rats by observing TER and permeability coefficients of FITC-BSA.

Results showed that, in the blank control group, the average TER of single layer of GECs was 60.6 ± 5.9 Ω*cm² and the average of permeability coefficient of FITC-BSA was 102 ± 2.6%. After 8 hours of AGEs incubation, the average TER of single layer of GECs gradually decreased and the average permeability coefficient of FITC-BSA gradually increased, with an increase of AGEs concentration. After AGEs stimulation at a concentration of 25 μg/mL, the monolayer cell TER decreased, significantly, which decreased to 68.3 ± 4.6% of the blank control group. It decreased gradually with an increase of AGEs. The average permeability coefficient of FITC-BSA was significantly increased to 149.4 ± 5.3% of the blank control group and the permeability coefficient of FITC-BSA was gradually increased with an increase of AGEs concentrations. After 8 hours of AGEs stimulation at a concentration of 100 μg/mL, the average TER was the lowest, 43.5 ± 4.1% of the blank control group, while the average FITC-BSA permeability was the highest. FITC-BSA average permeability was 217.6 ± 7.9% of the blank control group. Results indicate that the permeability of monolayer GECs increases with increasing concentrations of AGEs (Figures 3, 4).

**Influence of AGEs-HSA on GECs permeability in rats: different stimulus duration of AGEs-HSA (100 μg/mL) on permeability of GECs monolayer**

After third-generation GECs were used to construct monolayers of cells in the upper Transwell chambers, AGEs, at a concentration of 100 μg/mL, were chosen to stimulate incubation of GECs cells for 2 hours, 4 hours, and 8 hours, respectively. This study also detected the TER and FITC-BSA permeability coefficients to evaluate the permeability of GECs monolayers. Results showed that, in the blank control group, the average TER of GECs monolayer was 58.6 ± 5.1 Ω*cm² and the average permeability coefficient of FITC-BSA was 100 ± 5.8%. After incubation of GECs monolayers by AGEs at a concentration of 100 μg/mL, the average TER of GECs monolayers gradually decreased and the average permeability coefficient of FITC-BSA gradually increased, with prolonged stimulation times of AGEs. After AGEs were incubated for 2 hours, the TER of GECs monolayers decreased significantly to 70.2 ± 5.4% of the blank control.
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It still gradually decreased further with the increase of AGEs stimulation times. The average permeability coefficient of FITC-BSA increased significantly to 149.4 ± 6.6% of the blank control group and still increased gradually with the increase of AGEs stimulation time. After 8 hours of AGEs stimulation, the average TER was the lowest, 46.1 ± 5.4% of the blank control group. The FITC-BSA average permeability coefficient was the highest, 258.6 ± 7.3% of the blank control group. Results suggest that GECs monolayer permeability gradually increases with increases of AGEs stimulation times (Figures 5, 6).

Rac1 agonist (O-Me-cAMP) inhibit GECs permeability changes induced by AGEs-HSA

In the blank control group, the average TER of GECs monolayer was 59.3 ± 5.3 Ω·cm². The average of permeability coefficient of FITC-BSA was 100 ± 6.6%. After stimulation at a AGEs-HSA concentration of 100 ug/mL for eight hours, compared with the blank control group, the average TER was 27.8 ± 3.4 Ω·cm², decreasing significantly (P < 0.05), while the average of permeability coefficient of FITC-BSA was 189.32 ± 6.16%, increasing significantly (P < 0.05). After stimulation at a HSA concentration of 100 ug/mL for eight hours, the average TER was 63.6 ± 6.2 Ω·cm² and

Figure 3. Effects of different concentrations of AGEs-HSA on the average TER of GECs monolayer in rats after stimulation for the same time. TER decreased gradually with the increase of the AGEs. Note: #, control vs 25 ug/mL AGEs-HSA, P < 0.05.

Figure 4. Effects of different concentrations AGEs-HSA on the average FITC-BSA permeability coefficient of GECs monolayers in rats after stimulation for the same time. The permeability coefficient of FITC-BSA was gradually increased with the increase of AGESs. Note: #, control vs 25 ug/mL AGEs-HSA, P < 0.05.

Figure 5. Effects of different stimulating time of AGEs-HSA (100 ug/ml) on the average TER of GECs monolayers. The average TER of GECs monolayer gradually decreased with the prolonged stimulation time of AGEs. Note: #, control vs 100 ug/mL AGEs-HSA for 2 h.
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Table 1: Effects of different stimulating times of AGEs-HSA (100 ug/mL) on the FITC-BSA permeability coefficient of GECs monolayers. The average permeability coefficient of FITC-BSA gradually increased with prolonged stimulation times of AGEs. Note: #, control vs 100 ug/mL AGEs-HSA for 2 h, P < 0.05.

Figure 6. Effects of different stimulating times of AGEs-HSA (100 ug/mL) on the FITC-BSA permeability coefficient of GECs monolayers. The average permeability coefficient of FITC-BSA was 101 ± 6.9%, showing no statistical differences with the blank control group (P > 0.05). The average of permeability coefficients of FITC-BSA was 128.52 ± 3.53%, significantly decreasing (P < 0.05) (Figure 7). Results suggest that permeability changes of GECs monolayers caused by AGEs are effectively inhibited by O-Me-cAMP.

Effects of AGEs-HSA on Rac1 activity in GECs cells

Figure 7. Effects of Rac1 agonist on TRE of GECs monolayers induced by AGEs. Note: #, control vs AGEs-HSA, P < 0.05; *, O-Me-cAMP + AGEs-HSA vs AGEs-HSA, P < 0.05.

Figure 8. Effects of Rac1 agonist on the FITC-BSA permeability coefficient of GECs monolayer induced by AGEs. Note: #, control vs AGEs-HSA, P < 0.05; *, O-Me-cAMP + AGEs-HSA vs AGEs-HSA, P < 0.05.

Discussion

The major pathological manifestation of DN is glomerular capillary pathological changes, caused by a long-term high sugar environment. Early pathological physiological mechanisms include high glomerular filtration and an increase of glomerular capillary permeability [30, 31]. Glomerular filtration structure mainly includes GECs, basement membrane, and epithelial cells. GECs are the first barrier and play a key role. Therefore, it is reasonable to assume that the initial link of DN is damage to GECs structure and function, which causes the destruction of vascular endothelial cell connection, intercellular space, and filtration structure. The pathogenesis of microproteinuria caused by AGEs is effectively inhibited by O-Me-cAMP.
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by increased glomerular microvascular permeability is the main pathologic mechanism of early DN [10, 32, 33].

AGEs refer to excessive sugar and macromolecular substances, such as proteins, lipids, or nucleic acids, in the body. They spontaneously undergo chemical reactions without enzyme participation to form a stable covalent adduct. It was found that AGEs are closely related to the pathogenesis and development of many clinical diseases. Its formation and overaccumulation in diabetic patients were closely related to major complications of diabetes, such as nephropathy. Studies have shown that [34, 35], compared with normal people, the content of AGEs in diabetic patients is significantly increased. Its specific receptor is abundant in the kidneys, especially on the surface of glomerular microvascular endothelial cells. AGEs are involved in damage to GECs structure and function in DN. It manifests as morphological changes of GECs, leads to the destruction of various connection structures between GECs, an increase of intercellular fissures, and the formation of paracellular leakage, leading to increasing glomerular capillary permeability and proteinuria.

To study the influence of AGEs on GECs permeability, the original generation of GECs of rats was used as the object of study. Since GECs of rats and human GECs have many similarities, they can better simulate the influence of AGEs on renal glomerular microvascular endothelial cells in the human body. First, renal tissues of rats were treated. GECs of rats were isolated and cultured through mature experimental methods in the laboratory. GECs isolated from kidney tissues of rats were observed under an inverted microscope. They revealed polygonal or fusiform features, with pavers-like inlays, monolayer fusion growth, and contact inhibition. These conformed to the morphological and growth characteristics of human GECs. GECs were identified using the FITC-BSA binding test by fluorescein isothiocyanate. GECs were both identified by the methods of morphology and FITC-BSA, which laid a solid foundation for further research.

In this study, the GECs monolayer model was constructed on Transwell chambers to simulate the vascular endothelial cell barrier with glomerular filtration structure. The aim of this study was to explore the influence of AGEs on GECs permeability. After GECs monolayers were constructed, GECs monolayers in rats were stimulated and incubated by different concentrations of AGEs. Additionally, permeability changes of GECs monolayers were detected by TER and FITC-BSA permeability coefficients.

Results showed that AGEs decreased the average TER of GECs monolayer. The TER of GECs monolayers gradually decreased with increasing concentrations of AGEs-HSA. In contrast, AGEs increased the FITC-BSA permeability coefficients of GECs monolayers and permeability coefficients of FITC-BSA gradually increased with increasing concentrations of AGEs-HSA. TER and FITC-BSA permeability coefficients of GECs monolayers showed significant differences with the blank control group and the group stimulated by HSA alone. Results indicate that AGEs can increase the permeability of GECs monolayers. This effect is strengthened with the increase of AGEs concentrations, presenting a positive typical dose-dependent relationship. Diabetes patients with high blood glucose levels tend to progress to DN and promote AGEs formation. This will aggravate damage to renal glomerular endothelial cells. Therefore, it is necessary to actively control patient blood glucose levels to reduce and delay occurrence of DN.
On the other hand, after 2 hours, 4 hours, and 8 hours of respective incubation of GECs monolayers with the same concentration of AGEs, results showed that TER decreased gradually, while FITC-BSA permeability coefficients increased gradually, with the prolonging of stimulation times. As the same, both TER and FITC-BSA permeability coefficients of GECs monolayers showed significant differences with the blank control group and the group stimulated by HSA alone. Results suggest that AGEs increase the permeability of GECs monolayer. This effect is strengthened with the prolonging of AGEs stimulation times, presenting a positive typical time-dependent relationship. This is consistent with the duration of DN. The process from onset to occurrence of DN generally takes several decades of time. This may be related to the accumulation of damage to renal microvascular endothelial cells by AGEs.

To further study the mechanisms of AGEs effects on GECs permeability monolayers, the relationship between Rac signaling pathways and GECs permeability was discussed. Rac is a member of the Ras protein superfamily, including Rac1, Rac2, Rac3, and other subtypes. Currently, Rac1 has been extensively studied. Rac plays an important role in many different cellular physiological functions, such as cell morphology, movement of actin, transcriptional activation, and apoptotic signals [36-40].

In this study, the relationship between permeability and Rac1 signaling pathways of GECs monolayers in rats was explored with GECs as research objects. GECs pretreated with Rac1 specific agonist O-me-cAMP were used in the experiment. Results showed that Rac1 specific agonist O-me-cAMP significantly reduced the increase of GECs permeability induced by AGEs (P < 0.05). Changes of Rac1 activity levels in GECs after certain concentrations of AGEs-HSA stimulation, for a certain time period, were detected by pull-down assay. Results showed that Rac1 activity levels in GECs of the AGEs-HSA stimulation group were significantly reduced, compared with the blank control group and HSA alone group (P < 0.05). This study further proved that Rac1 signals are involved in GECs morphological and functional changes caused by AGEs.

In addition, this research found that O-me-cAMP failed to completely reduce the permeability of GECs to normal levels, still showing significant differences, compared with the control group (P < 0.05). Results suggest that there may be other signaling pathways, besides Rac1 signaling pathways, participating in changing GECs morphology and function. This provides direction for the next study.

**Conclusion**

The permeability of rat GECs cell monolayers increases with concentrations of AGEs, showing dose-dependence and time-dependence. Rac1 signaling pathways play an important role in morphological and functional changes of GECs mediated by AGEs. Discovery of other signaling pathways participating in changing GECs morphology and function requires further investigation.

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**Disclosure of conflict of interest**

None.

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**References**


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[26] You QH, Gao L, Yue Y and Sun GY. Methods for measurement of permeability coefficient of pulmonary microvascular endothelial cells in
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[27] Li AF, Tane N and Roy S. Fibronectin overexpression inhibits trabecular meshwork cell monolayer permeability. Mol Vis 2004; 10: 750-757.


[34] Yamagishi S. Role of advanced glycation end products (AGEs) and receptor for AGEs (RAGE) in vascular damage in diabetes. Exp Gerontol 2011; 46: 217-224.


