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The role of polyol pathway in high glucose-induced endothelial cell damages

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Abstract

To clarify the mechanism by which hyperglycemia in diabetes mellitus causes endothelial cell damages, the effects of high glucose on DNA fragmentation and caspase-3 activity of cultured endothelial cells and on the generation of 8-hydroxy-2′-deoxyguanosine (8-OHdG) were studied. Furthermore, the involvement of the polyol pathway in this process was investigated using aldose reductase inhibitor (SNK-860).

Human umbilical vein endothelial cells (HUVECs) were incubated with 5.5 mmol/L (low glucose medium) or 28 mmol/L (high glucose medium) of glucose. The amounts of fragmented DNA, caspase-3 activity and 8-OHdG in the medium increased in significantly greater extent in high glucose-incubated HUVECs than in low glucose-incubated HUVECs. No significant increase in fragmented DNA or 8-OHdG was observed when HUVECs were incubated with mannitol (500 mg/mL). The concentration of intracellular sorbitol was significantly higher in HUVECs incubated in high glucose medium than that in low glucose medium. Addition of the aldose reductase inhibitor SNK-860 dose-dependently decreased the intracellular sorbitol concentration in HUVECs incubated in high glucose medium, and also significantly suppressed the increases in fragmented DNA, caspase-3 activity and 8-OHdG by conditioning with high glucose medium.

These results suggest that high glucose-induced endothelial cell damages may be mediated by activation of the polyol pathway accompanied by augmented oxidative stress.

Keywords: Polyol pathway; Endothelial cell damage; High glucose; Aldose reductase inhibitor; Oxidative stress

1. Introduction

Diabetes mellitus is known to be an important risk factor of myocardial infarction in addition to organ injuries such as retinopathy and nephropathy [1,2]. Endothelial cell damage is among the most common injuries involved in these vascular complications.

Endothelial cells play a role in anticoagulation effect, and also control vasoconstriction. Failure of the anti-coagulant effect causes thrombus formation, leading to occlusion of microvessels observed in the early stage of diabetic retinopathy and also acute coronary syndrome.

Various mechanisms of cell injuries in diabetes mellitus have been reported, including accelerated glycation [3], increased protein kinase C activity [4] and increased oxidative stress [5], but the precise mechanism is not fully understood. Hotta's group [6–8] proposed the involvement of the polyol pathway as a mechanism of various organ injuries induced by high concentration of glucose. The polyol pathway consists of two steps; the first is the conversion of glucose to

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sorbitol and the second is the conversion of sorbitol to fructose. The key enzyme is aldose reductase that converts glucose to sorbitol [9–11]. This enzyme is found in many tissues [7,12–15]. Hyperglycemia enhances the polyol pathway [16], resulting in accumulation of sorbitol in the cells [17]. Accumulation of sorbitol in cells causes various organ injuries [18]. High osmotic pressure [18,19] and high oxidative stress have been proposed [20] as the mechanisms by which polyol pathway is involved in cell injury. Based on these findings, aldose reductase inhibitor was synthesized and used clinically for the prevention of diabetic peripheral polyneuropathy. However, the precise mechanism of polyol pathway is not yet fully understood.

Recently, apoptosis in which DNA is primarily injured is known to cause the most formidable cell injury. One of apoptosis-inducing mechanism is oxidative stress. Oxidative stress causes denaturation of proteins and lipids. The role of the polyol pathway in oxidative stress has been reported [20–22], but is still unclear in the endothelial cells. Recently, a potent aldose reductase inhibitor, SNK-860 has been developed [23].

In this study, to clarify whether endothelial cell damage induced by high glucose concentration is mediated by the polyol pathway, and how cell injury occurs via the polyol pathway, we studied the effects of high glucose concentration on DNA fragmentation, caspase-3 activity and oxidative product as well as the sorbitol concentrations in cultured human umbilical vein endothelial cells. We also examined the inhibitory effects of the aldose reductase inhibitor SNK-860 on the above parameters.

2. Materials and methods

2.1. Endothelial cell culture

Endothelial cells were derived from human umbilical vein (HUVECs), and were cultured as previously described [24]. In all experiments, HUVECs were cultured in modified MCDB-131 medium (EBM, Clonetics) supplemented with 2% FBS, gentamicin (50 μg/mL), bovine brain extract (12 μg/mL), hydrocortisone (1 μg/mL), and human epidermal growth factor (10 ng/mL). HUVECs were grown to confluence at 37 °C in a humidified atmosphere containing 5% CO_2. Cultures of the third passage were used in the experiments. HUVECs were incubated in culture medium containing 5.5 or 28 mmol/L of glucose. The effects of glucose concentration in medium on DNA fragmentation, caspase-3 activity and 8-OHdG were evaluated after incubation in normal glucose (5.5 mmol/L) or high glucose (28 mmol/L) medium for 120 h. Mannitol was used as osmotic pressure-matched control for the high glucose medium. To evaluate the effects of the aldose reductase inhibitor SNK-860 on the above parameters, after 72-h incubation in medium containing normal or high glucose concentration, HUVECs were incubated in the presence or absence of SNK-860 for 48 h. SNK-860 was kindly provided by Sanwa Kagaku Kenkyusho (Nagoya, Japan).

2.2. DNA fragmentation

The DNA of cells in culture flasks was labeled with 5-bromo-2′-deoxy-uridine (BrdU) for 24 h. After incubation for specified periods of time, the amount of BrdU-labeled DNA released into the supernatant from dead cells was measured by the quantitative sandwich enzyme immunoassay (Cellular DNA Fragmentation ELISA, Boehringer Mannhein, Cat. no.1585045). Data was corrected using the quantity of total DNA in the well.

2.3. Measurement of sorbitol content in endothelial cells

Endothelial cells were homogenized by sonication in 2 mL of cold water for 30 s. For sorbitol measurement, 1 mL of trichloroacetic acid (10% final concentration) was added to the homogenate to remove proteins, and centrifuged at 10,000 × *g* for 5 min. The supernatant fraction was derived to sorbitol acetate derivative according to the method of Guerrant and co-workers [25]. Thereafter, the sorbitol quantity was determined by high performance liquid chromatography (HP1050, Hewlett Packard, Palo Alto, CA, USA) with mass spectrometer (TSQ, Finnigan Mat, San Jose, CA, USA) using Cadenza CD-C18 column (100 mm × 2.0 mm i.d., 3 μm, Imtakt, Kyoto, Japan). Data was corrected by the quantity of total protein in the well, which was measured by bicinchoninic acid protein assay [26].

2.4. Measurement of caspase-3 activity

Activity of caspase-3 was measured using a commercially available kit (CaspACE™ Assay System, Promega) following the instructions of the manufacturer. Cells were centrifuged at 200 × *g* for 10 min, and cell pellets were kept on ice. The cell pellets were washed with ice-cold PBS and suspended in cell lysis buffer. Cell lysates were incubated on ice for 15 min. After centrifugation at 15,000 × *g* for 20 min, the supernatants were collected and used as samples. An equal volume of assay reagent DEVD-pNA and a fluorometric substrate of caspase-3 were added to the samples, and incubated in 96-well plates at 37 °C for 4 h. The fluorescence of cleaved pNA was measured on an automatic microtiter plate reader (Microplate Reader EZS-ABS, Asahi Techno Glass Co.) by spectrofluorometery at an excitation wavelength of 405 nm.

2.5. Measurement of 8-hydroxy-2′-deoxyguanosine

Cells were centrifuged at 200 × *g* for 10 min. Cell pellets were washed with saline, and disrupted with a high molecular weight buffer, pH 7.5 (150 μmol/L NaCl, 10 μmol/L