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Cariporide inhibits high glucose-mediated adhesion of monocyte–endothelial cell and expression of intercellular adhesion molecule-1

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Abstract

The aim of this study was to examine whether cariporide, a new inhibitor of Na^+/H^+ exchanger 1 (NHE-1), may inhibit high glucose-induced monocyte–endothelial cell adhesion and the expression of intercellular adhesion molecule-1 (ICAM-1). Cultured endothelial cells were incubated with normal glucose control (5.5 mM), cariporide control (5.5 mM glucose plus 10 μM cariporide), hyperosmolarity (5.5 mM glucose plus 16.5 mM mannitol), high glucose (HG, 22 mM), low-concentration cariporide (22 mM glucose plus 0.1 μM cariporide), medium-concentration cariporide (22 mM glucose plus 1 μM cariporide), and high-concentration cariporide (22 mM glucose plus 10 μM cariporide) for 24 h. Monocytes were isolated from peripheral human blood. Adhered monocytes were quantified by measuring their protein content. ICAM-1 expression and NHE-1 activity was determined with enzyme-linked immunosorbent assay (ELISA) and pH-sensitive fluorescent spectrophotometry. Exposure of endothelial cells to HG for 24 h caused an increase of adhesion of monocytes to endothelial cells and an increased expression of ICAM-1. However, these effects were reversed by treatment with cariporide (0.1, 1, 10 μM) in a concentration-dependent manner. Furthermore, cariporide (1 μM) was able to inhibit the activation of NHE-1 induced by HG in endothelial cells. These findings suggest that cariporide might inhibit HG-mediated monocyte–endothelial cell adhesion and expression of ICAM-1 by inhibiting the activation of NHE-1.
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Keywords: Adhesion; Cariporide; Endothelial cell; Glucose; ICAM-1

Introduction

Previous epidemiological investigations have shown that diabetes mellitus constitutes one of the major risk factors for atherosclerotic cardiovascular diseases such as coronary heart disease, hypertension and stroke (Selvin et al., 2005; Schmidt et al., 2005; Abel, 2005; Veiraiah, 2005). Several mechanisms have been proposed to explain why diabetic patients are at an increased risk for such vascular disorders, such as an excessive concentration of glucose causing glycation of various proteins, a decrease in the level of oxygen dissociation in erythrocytes (Yamagishi et al., 2005), an increase in platelet aggregation (Mustard and Packham, 1984), an increase in the level of very low density lipoproteins and a decrease in high density lipoprotein cholesterol (Schonfeld, 1985). However, the mechanism is still unclear.

One of the earliest events in atherogenesis is an increased binding of monocytes to endothelial cells and the entry of monocytes into the vessel wall (Ross, 1999). Studies have identified three molecules, intercellular adhesion molecule-1 (ICAM-1), E-selectin and vascular cell adhesion molecule-1 (VCAM-1) that are involved in the molecular mechanism of the process (Omi et al., 2002; Bannan et al., 1998; Ceriello et al., 1998). These adhesion molecules can be induced on the endothelial surface and can support the adhesion of various leukocytes. Of these, ICAM-1 has been shown immunohistochemically to be expressed in human atherosclerotic plaques and to play an important role in mediating the localization of monocytes in the intima of arteries (Poston et al., 1992).

Subtype 1 of Na^+/H^+ exchanger 1 (NHE-1), which predominates in the mammalian cardiovascular system, is a

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ubiquitous integral membrane protein and thought to play an important role in the regulation of cell volume, intracellular pH, and intracellular Na^{+}/Ca^{2+} concentration. Inhibition of NHE-1 activity has shown many cardio-protective effects against myocardial ischemia–reperfusion injury (Stromer et al., 2000), myocardial hypertrophy (Cingolani, 1999), and diabetes nephropathy (Jandeleit-Dahm et al., 2000). Furthermore, it has been demonstrated that Na^{+}/H^{+} exchange inhibitor, SM-20220, attenuates leukocyte adhesion induced by ischemia–reperfusion (Horikawa et al., 2001). Our previous experiments have demonstrated that cariporide, a new NHE-1 inhibitor, prevented endothelial dysfunction induced by high glucose (Wang et al., 2005).

Based on these results, it is possible that NHE-1 will play an important role in high glucose-mediated adhesion of monocytes to endothelial cells. Accordingly, the aim of this study is to evaluate whether cariporide may inhibit both monocyte–endothelial cell adhesion induced by high glucose and intercellular adhesion molecule-1 expression.

Materials and methods

Materials

Cariporide was kindly provided by Hoechst (Frankfurt, Germany). Hydroxyethyl piperazine ethanesulfonic acid (HEPES), nigericin, 2-carboxyethyl-5(6)-carboxyfluorescein (BCECF), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), endothelial cell growth factor (ECGF), Ficoll–Paque, dextran, trypsin and monoclonal antibody for ICAM-1 were purchased from Sigma Chemical Co, USA. The kit for ELISA was purchased from Zhongshan Biochemical Company, Beijing, China. All other chemicals were of reagent grade.

Endothelial cell culture

The human umbilical vein endothelial cell (HUVEC) line was purchased from ATCC (CRL-1730). The HUVECs were grown in DMEM supplemented with 10% FBS, 12.5 mg/ml ECGF, 1 mg/ml hydrocortisone, 100 U/ml penicillin and 100 mg/ml streptomycin. The cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Culture medium was replaced twice a week, and cells were subcultured when confluent. Cultures were expanded by brief trypsinization using 0.25% trypsin in phosphate-buffered saline (PBS) containing 0.02% EDTA. Cells at passage 4 were used for all experiments.

Experimental protocol

The study was carried out on 7 groups: normal glucose control (5.5 mM), cariporide control (5.5 mM glucose plus 10 μM cariporide), hyperosmolarity (5.5 mM glucose plus 16.5 mM mannitol), high glucose (HG, 22 mM), low-concentration cariporide (22 mM glucose plus 0.1 μM cariporide), medium-concentration cariporide (22 mM glucose plus 1 μM cariporide), and high-concentration cariporide (22 mM glucose plus 10 μM cariporide). HUVECs were incubated with medium as described above for 24 h.

Monocyte isolation from peripheral human blood

The monocyte isolation from peripheral human blood was described previously (Goldrosen et al., 1997). About 100 ml of the buffer coat with white blood cells from the blood center was divided into sterile 50-ml tubes (max of 20 ml for a 50-ml tube) with aliquot. An equal volume of PBS and 10% of the volume of 6% dextran were added to the tubes and mixed by inverting the tube a few times. The mixtures were allowed to stand at room temperature (RT) for 35 min. Then the upper phase was removed and transferred to two 50-ml tubes. The tubes were filled up to 50 ml with PBS and centrifuged at 1000 rpm for 10 min. Pellets were resuspended in 30 ml PBS, which was transferred to two new 50-ml tubes (15 ml/tube). About 10 ml of Ficoll–Paque was added underneath the 15 ml cell suspensions using a 20-ml syringe with a thick needle. The tubes were centrifuged at 1500 rpm for 35 min. During centrifugation, the monocytes formed a white band at the interface between the lower and upper steps of the gradient. The white layer was collected to a new 50-ml tube which was filled with PBS and centrifuged at 1500 rpm for 35 min. The resulting pellet was resuspended in DMEM supplemented with 10% FBS. The cells were counted and trypan blue exclusion indicated that the cells were more than 95% viable. Then the monocytes were used for monocyte–endothelial cell adhesion assay.

Monocyte–endothelial cell adhesion assay

The monocyte–endothelial cell adhesion assay was described previously (Pruefer et al., 1999). Briefly, 1×10^{7} HUVECs/well were seeded into 48-well plates, and incubated for 24 h at 37 °C in the presence or absence of various test agents. After treatment, culture medium was replaced with PBS, and then 1×10^{5} monocytes/well were added and allowed to adhere for 60 min at 37 °C. The monolayers were gently washed twice with PBS to remove non-adhered monocyte and adhered monocytes were quantified by protein-quantitation methods. Monocyte adherence was expressed as the ratio of the protein-quantitation of adhered monocytes to that of the total monocytes (1×10^{5} cells) added.

Assay of expression of ICAM-1 by ELISA

The protocol used for cell ELISA of ICAM-1 was modified from that of Rothlein et al. (1988). Briefly, HUVECs in 96-well microplates, after being treated with various test agents, were washed with warm Hanks' balanced salt solution (HBSS) containing 0.1% bovine serum albumin (BSA). The monolayers were washed and then incubated with mouse anti-human ICAM-1 monoclonal antibodies at a final concentration of 0.5 mg/ml in HBSS containing 0.1% BSA to detect the surface expression of ICAM-1. After incubation of cells at RT for 30 min, the plates were washed 5 times with HBSS containing

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