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Insulin enhances dendritic cell maturation and scavenger receptor-mediated uptake of oxidised low-density lipoprotein

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ABSTRACT

Objectives: The prevalence of atherosclerotic cardiovascular disease is increased in patients with type 2 diabetes. The role of hyperinsulinaemia as an independent participant in the atherogenic process is controversial. Therefore, we examined whether insulin regulates the expression of scavenger receptors responsible for oxidised low-density lipoprotein (oxLDL) uptake in dendritic cells (DCs). In addition, we investigated the impact of insulin on DC maturation with regard to changes in phenotype and cytokine secretion.

Methods: Immature DCs were cultured with different concentrations of insulin (1 nmol/L, 10 nmol/L, 50 nmol/L, and 100 nmol/L) in the absence or presence of LY294002 or PD98059 for 24 h. The expression of the scavenger receptors SR-A and CD36 was determined by real-time PCR and Western blot analysis. Furthermore, DCs were incubated with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI)-labelled oxLDL. The DiI-oxLDL-incorporated fraction was investigated by flow cytometry. Finally, flow cytometry was used to investigate immunophenotypic protein expression (CD83, CD86, and CD11a). Supernatant cytokine measurements were used as indicators of immune function.

Results: The incubation of DCs with insulin enhanced SR-A and CD36 gene and protein expression in a dose-dependent manner. This effect was partially abolished by PD98059, which is an extracellular signal-regulated kinase (ERK) inhibitor. However, LY294002 did not inhibit the effect of insulin on scavenger receptor expression. A high concentration of insulin increased the oxLDL-uptake capacity of DCs. Inhibition of the scavenger receptors SR-A and CD36 significantly reduced oxLDL uptake. Furthermore, a high concentration of insulin induced DC maturation. The pro-atherosclerotic chemokines IL-6 and IL-12 were induced by a high concentration of insulin, whereas the release of anti-atherosclerotic IL-10 was reduced.

Conclusion: This study suggests that hyperinsulinaemia can promote DC activation and up-regulate the expression of the scavenger receptors SR-A and CD36, which can increase the oxLDL-uptake capacity of DCs. The results of the present study indicate that one of the mechanisms by which insulin promotes atherogenesis is mediated by its effects on DCs.

1. Introduction

Cardiovascular diseases remain the leading cause of death in adults with type 2 diabetes mellitus, which is characterised by insulin resistance and compensatory hyperinsulinaemia (Haffner, Lehto, Ronnemaa, et al., 1998; Lusis, 2000; Marray & Lopez, 1997). The causative role of insulin resistance in cardiovascular disease has been proven both epidemiologically and experimentally (DeFronzo, 2006; Hanley, Williams, Stern, et al., 2002; Rutter, Meigs, Sullivan, et al., 2005), but minimal evidence supporting a direct cause-and-effect relationship between hyperinsulinaemia and atherosclerosis is available. Furthermore, the role of hyperinsulinaemia as an independent risk factor is controversial. Several prospective studies of non-diabetic and diabetic patients, including the Quebec Cardiovascular study, have demonstrated an association between hyperinsulinaemia resulting from insulin resistance and atherosclerotic disorders (Ducimetiere, Eschwege, Papoz, et al., 1980; Haffner, Stern, Hazuda, et al., 1986; Pyörälä, Savolainen, Kankola, et al., 1985); however, other studies, such as that of Welin and colleagues, failed to demonstrate such an association (Ferrara, Barrett-Connor, & Edelstein, 1994; Welin, Eriksson, Larsson, et al., 1992). Because hyperinsulinaemia typically occurs in states of insulin resistance, it is difficult to determine whether it plays an independent role in the pathogenesis of atherosclerosis.

Competing interests: The authors declare that they have no conflicts of interest.

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Atherosclerosis is a chronic inflammatory disease of the arterial vessel wall, and immune responses to autoantigens or crossreactions with foreign antigens play a crucial role in its initiation and progression (Hansson, 2005; Perrins & Bobryshev, 2011). Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that present various exogenous and endogenous antigens to T lymphocytes, providing an important link between innate and adaptive immune responses. Accumulating evidence has suggested that DCs are actively involved in the process of atherosclerosis by activating T cells and stimulating vascular inflammation (Bobryshev, 2005). The uptake of oxidised low-density lipoprotein (oxLDL) through a variety of scavenger receptors by macrophages and monocyte-derived DCs is a critical step in atherosclerosis initiation and progression. The stimulation of DCs by oxLDL through binding to scavenger receptors leads to their activation and is accompanied by enhanced cytokine production (Nickel, Schmauss, Hanssen, et al., 2009). Furthermore, although lipid uptake and foam cell formation in arteries have been attributed mainly to macrophages, recent studies have demonstrated that DCs in the subendothelial space of the aorta can also efficiently accumulate lipids and differentiate into foam cells, thereby contributing to the initiation and further progression of atherosclerosis (Paulson, Zhu, Chen, et al., 2010). The essential scavenger receptors SR-A and CD36 have been shown to be involved in oxLDL uptake and foam cell formation.

Recent studies have demonstrated that elevated insulin increases the expression of scavenger receptors in macrophages, thereby contributing to diabetes and its related disease atherosclerosis (Kashyap, loachimescu, Gornik, et al., 2009; Park, Kashyap, Major, et al., 2012). However, to the best of our knowledge, little is known about how insulin affects the expression of scavenger receptors in DCs. Therefore, we examined whether insulin regulates scavenger receptor expression in DCs, focusing on CD36 and SR-A, and whether insulin modulates maturation and differentiation processes in these cells.

2. Methods

2.1. Materials

Human CD14 + immunomagnetic microbeads were obtained from Miltenyi Biotech GmbH (Bergisch-Gladbach, Germany). Recombinant human granulocyte/macrophage colony-stimulating factor (rhGM-CSF); recombinant human interleukin-4 (rhIL-4); enzyme-linked immunosorbent assay (ELISA) kits for IL-6, IL-10, and IL-12p70; and tumour necrosis factor-α (TNF-α) were obtained from R&D Systems (USA). Histopaque-1077 and TRIzol reagent were purchased from Invitrogen (USA). The reverse transcription system GoTaq qPCR Master Mix, PD98059, and LY294002 were obtained from Promega (USA). Insulin was purchased from Sigma (USA). Goat anti-human SR-A polyclonal antibody and mouse anti-human CD36 monoclonal antibody were purchased from Santa Cruz Biotechnology. Mouse anti-human CD83-FITC, CD86-FITC, and CD11a-FITC antibodies were obtained from BD Pharmingen. In addition, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI)-oxLDL was purchased from Peking Union-Biology Co. Ltd.

2.2. Generation of monocyte-derived dendritic cells

Peripheral blood mononuclear cells (PBMCs) were obtained from healthy volunteers. Briefly, blood was diluted 1:2 in PBS layered over Histopaque 1077 and centrifuged for 30 min at 2,000 rpm at room temperature. The interface was recovered and washed thrice in PBS. CD14 + PBMCs were purified using CD14 + immunomagnetic microbeads and incubated in RPMI-1640 medium supplemented with GM-CSF (100 ng/mL) and IL-4 (50 ng/mL) in six-well tissue culture plates at 37 °C and 5% CO2. The medium was replaced every 2 days. On day six, the cells were exposed to various concentrations of insulin (1 nmol/L, 10 nmol/L, 50 nmol/L, and 100 nmol/L) for an additional 24 h. In some experiments, 10 μmol/L of LY294002 or 50 μmol/L of PD98059 was added simultaneously with 100 nmol/L of insulin.

2.3. Real-time PCR

The mRNA expression of the different scavenger receptors (SR-A and CD36) in DCs was analysed using real-time quantitative reverse transcription polymerase chain reaction (RT-PCR). Total RNA was isolated and treated with DNase using a TRIzol Reagent Kit according to the manufacturer's instructions. Five micrograms of total RNA was reverse-transcribed using oligo-dT and SuperScript III. SR-A was amplified using the sense primer 5'-TCCTCGTGTTTGCAGTTCTC-3' and antisense primer 5'-GCAATTCTTCGTTTCCCACT-3'. CD36 was amplified using the sense primer 5'-CGCTGAGGACAACACAGTCT-3' and antisense primer 5'-GTTGTCAGCCTCTGTTCCAA-3'. LOX-1 was amplified using the sense primer 5'-GGGCTCATTTAACTGGGAAA-3' and antisense primer 5'-GAAATTGCTTGCTGGATGAA-3'. Quantitative PCR using SYBR Green reagent was performed with an ABI 7500 Real-time PCR System (Applied Biosystems, USA). Gene expression was analysed by the system software, and the number of copies of each mRNA molecule was determined using the standard curve method.

2.4. Western blotting

After treatment with insulin, DCs were washed with ice-cold PBS and then lysed in cold cell lysis buffer. Protein concentrations were measured using a BCA Protein Assay Reagent Kit (Beyotime Institute of Biotechnology). Protein extracts (20 μg) were separated by 7.5% SDS-PAGE, transferred to a nitrocellulose membrane by electrotransfer (200 V for 30–60 min), and blocked with 5% non-fat milk for 1 h at room temperature. Antigen–antibody complexes were detected using an appropriate HRP-labelled secondary antibody with an ECL detection system (Thermo Fisher Scientific, Rockford, IL, USA) according to the manufacturer's protocol. The resulting bands were analysed densitometrically using ImageQuant software (Molecular Dynamics, Sunnyvale, CA, USA). All values were normalised to a tubulin loading control.

2.5. oxLDL uptake

After treatment with 10 nmol/L or 100 nmol/L insulin for 24 h, DCs were incubated with DiI-labelled oxLDL (10 μg/mL) for 60 min at 37 °C. Then, the cells were harvested and washed thrice at 4 °C. The DiI-oxLDL-incorporated fraction was quantified by flow cytometry.

2.6. Inhibition of oxLDL uptake

DCs were incubated with DiI-oxLDL (10 μg/mL) for 4 h at 4 °C with or without anti-SR-A- (Biozol, USA) and anti-CD36- (Abcam, USA) neutralising antibodies. The endocytosed DiI-oxLDL fraction was analysed by flow cytometry. To eliminate nonspecific blocking, control IgG antibodies were used.

2.7. Flow cytometric measurement

On culture day 6, DCs were exposed to 1 nmol/L, 10 nmol/L, and 100 nmol/L for 24 h. The cells (1×10^6) were harvested, washed, and resuspended in ice-cold PBS containing 5% foetal bovine serum to prevent nonspecific binding and incubated with FITC-conjugated mAbs (CD83-FITC, CD86-FITC, and CD11a-FITC) for 30 min at 4 °C. Then, the cells were analysed via flow cytometry (Becton Dickinson). Cells stained with the appropriate isotype-matched Ig were used as negative controls.