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Resistin and high glucose induce ICAM-1, P-selectin and fractalkine in human endothelial cells

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Background and aims: Atherosclerosis is accelerated in diabetic conditions. Clinical data, and in vivo and in vitro studies suggest that endothelial dysfunction play a key role in this process and that cell adhesion molecules, cytokines and chemokines are actively involved. Resistin, a recently described cytokine, has an established role in mice as a link between obesity and diabetes but an unclear function in humans was it is assumed to be primarily involved in inflammation. Recent clinical data indicate that plasma resistin levels are increased in patients with diabetes complicated with atherosclerosis, but the involved mechanisms are not very well known. To better understand resistin role in these conditions, we performed studies on resistin and high glucose (as inducers of accelerated atherosclerosis in diabetic conditions) direct action on human endothelial cells (HEC). The aims of our studies were: i) to investigate the effects of resistin and high glucose in ICAM-1, P-selectin and fractalkine expression in HEC; ii) to reveal the signaling pathways possible involved.

Materials and methods: Human endothelial cells (Eahy926 cells) in culture were exposed to resistin (50 or 100ng/ml), high glucose (33mM) or resistin and high glucose for 6,18, 24 and 48 hours. The expression of cell adhesion molecules ICAM-1, P-selectin and fractalkine was detected by RT-PCR (gene expression) and by Elisa and Western blot (protein expression). To identify resistin and high glucose downstream signaling pathways the activation of p38, and JNK MAPK and of transcription factors NF-kB and AP1 was determined (western blot). In addition, intracellular accumulation of reactive oxygen species (ROS) in HEC was monitored by fluorimetry using 2',7'-dichlorofluorescin diacetate (DCFH-DA).

Results: Our data show that resistin or high glucose induced: i) an increase in gene and protein expression of ICAM-1, P-selectin and fractalkine; ii) an enhancement of the nuclear level of p65 and pc-fun proteins; iii) an increase in the intracellular level of ROS; iv) an increased activation of JNK and p38 MAPK (in stimulation with resistin and high glucose); i) induced ICAM-1, P-selectin and fractalkine at levels that do not exceed the levels of every inducer alone; ii) activated p38 and JNK MAPK at similar levels as every inducer alone and iii) increased intracellular ROS levels at similar levels as every single inducer.

Conclusion: Our results suggest that resistin, high glucose or resistin and high glucose activate human endothelial cells by inducing the cell adhesion molecules ICAM-1, P-selectin and fractalkine and MAPK, ROS and the transcription factors NF-kB and AP-1 may be involved in the activation process. Supported by: Romanian Academy and Ministry of Research and Education-National Center of Grants Management

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Effect of consumption of conjugated linoleic acids on the LDL induced expression of adhesion molecules on endothelial cells

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Background and aims: Increased expression of adhesion molecules, such as the intracellular adhesion molecule (ICAM-1), is associated with an activated endothelium. Adhesion molecules promote the migration of leucocytes into the intima, an early step in atherogenesis. Further, elevated levels of soluble adhesion molecules are frequently observed to be a risk marker for the development of type 2 diabetes. This study focused on conjugated linoleic acids (CLA) and their effect on the LDL-induced expression of ICAM-1 on endothelial cells.

Materials and methods: In a randomized, double blind, crossover study, 20 healthy males, age 50 to 75 years, with an increased body mass index (BMI: 25-30 kg/m2) consumed 4.25g/d of either linoleic acid (LA) or cis-9, trans-11 CLA isomer or the trans 10, cis-12 CLA isomer or a 50:50 mix of both isomers for 4 weeks. 4-week treatments were separated by 4-6 week washout periods. Blood samples were drawn in the fasting state and 5h after ingestion of a liquid fat- and sucrose-rich test meal plus 3 capsules of the respective supplement (2.125g). Fasting and postprandial LDL were isolated by ultracentrifugation and adjusted to a protein concentration of 0.3 mg/ml. Aliquots were oxidized with copper (2 or 5 µM Cu, 18h).

Human umbilical vein endothelial cells (HUVECs) were incubated for 16h with native LDL as well as oxidized LDL. The expression of ICAM-1 was determined by an enzyme-linked immunosorbent assay (ELISA). Cytotoxicity induced by the incubation of HUVECs with LDL was assessed from the release of lactate dehydrogenase (LDH) into the culture media. Statistical analysis of the results was carried out by a multi-factor analysis of variance (MANOVA) followed by post hoc Bonferroni. Data given are means ± SEM.

Results: Oxidized LDL induced expression of ICAM-1 more than native LDL, and expression was higher with higher degree of oxidation (2 or 5 µM Cu). LDL isolated from subjects after consumption of trans-10, cis-12 CLA-stimulated ICAM-1 less than LDL after LA intervention (Bonferroni, P<0.05). ICAM expression after consumption of the CLA mix and cis-9, trans-10 CLA intervention tended to be lower than after LA consumption. Interestingly, postprandial LDL did not increase the ICAM-1 expression beyond that of fasting LDL, but they were more cytotoxic (Bonferroni, P<0.05). LDL obtained after CLA mix intervention were more cytotoxic than after all other interventions (Bonferroni, P<0.05). But overall the cytotoxic effects were low.

Conclusion: LDL obtained after CLA-enriched diets, in particular after consumption of the trans-10, cis-12 CLA isomer, decreased expression of ICAM-1 in endothelial cells compared to LA. This indicates that CLA may attenuate inflammatory processes and thus act in a beneficial manner.

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Centella Asiatica inhibits TNFα-induced adhesion molecule expression in endothelial cells of umbilical cords from gestational diabetic women

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Background and aims: Diabetes mellitus is associated with inflammatory endothelial activation and increased vascular leukocyte adhesion molecules expression, both playing a prominent role in the development of vascular complications. Centella Asiatica (CA) has shown anti-inflammatory properties in a variety of experimental models; however, its effects on vascular adhesion molecule synthesis and exposure have not yet been tested. Thus, we evaluated the effect of CA on TNFα-stimulated adhesion molecule expression in cultured endothelial cells obtained from umbilical cords of gestational diabetic and control women.

Materials and methods: Human Umbilical Vein Endothelial Cells (HUVEC) obtained at delivery form umbilical cords of 10 healthy women (C) and 10 women with gestational diabetes (GD) were stimulated with TNFα (1 ng/mL) after a 48 hours pre-incubation with CA (25 µg/mL) or without buffer. After 12 and 16 hours, vascular cell adhesion molecules (VCAM-1), intercellular cell adhesion molecules (ICAM-1) and E-Selectin protein levels (Western Blot) and their surface expression (biparametric flow cytometry analysis) were assessed. The functional consequences of C- and GD-HUVEC treatment with CA on VCAM-1 membrane exposure were also evaluated by human monocyte cell (U937 line) adhesion assay.

Results: After a 12 hours TNFα stimulation, VCAM-1, ICAM-1 and E-Selectin protein levels were significantly higher in GD- as compared in C-HUVEC (p<0.05, Western Blot analysis). Preincubation with CA significantly decreased the effects of 12 hours TNFα-stimulation on VCAM-1 protein levels in GD-HUVEC (25-30%), while no effect was observed on C-HUVEC. Flow cytometry analysis demonstrated that, following CA pre incubation, the percentage of cells positive for surface VCAM-1 and ICAM-1 expression was modestly but significantly lower both in C- and GD-HUVEC after 12 and 16 hours TNFα stimulation. In addition, as compared to cells not pre-exposed to CA, both VCAM-1 and ICAM-1 MFI ratio (Mean Fluorescence Intensity) was lower in both CA-preincubated C- and GD-HUVEC after 12 (MFI R