

The β -Specific Protein Kinase C Inhibitor Ruboxistaurin (LY333531) Suppresses Glucose-Induced Adhesion of Human Monocytes to Endothelial Cells *in Vitro*

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Abstract

Aims:

Strong evidence shows that late diabetic complications in diabetes mellitus are substantially related to an increased synthesis of diacylglycerol with a subsequent activation of protein kinase C (PKC) β . Several studies have shown that specific inhibition of the PKC isoform β by ruboxistaurin is able to attenuate the development of microvascular complications under diabetic conditions. The aim of this *in vitro* study was to investigate the effect of ruboxistaurin on glucose-induced adhesion of monocytes to endothelial cells, representing one of the first pivotal steps in the course of atherogenesis.

Methods:

Human umbilical venous endothelial cells were isolated and cultured to confluence in microtiter plates. After coinubation with monocytes in the presence of 0, 10, or 400 ng ruboxistaurin to achieve PKC β -specific and -unspecific PKC inhibition, cells were fixed and monocyte adhesion was determined by means of a standardized chemiluminescence assay. Expression of adhesion molecules (intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin) was also measured by chemiluminescence methods.

Results:

Adhesion of monocytes to endothelial cells cultured under hyperglycemic conditions (27.7 mM glucose) was increased by $30.9 \pm 5.1\%$ ($p < 0.001$) versus endothelial cells cultured under normoglycemic (NG) conditions (5.5 mM). Pretreatment of endothelial cells with 10 nM (PKC β -specific concentration) and 400 nM (PKC β -unspecific concentration) led to a significant reduction of glucose-induced adhesion of monocytes to endothelial cells that was statistically not different from endothelial adhesion under NG conditions (-7.2 ± 3.1 and $-8.1 \pm 2.6\%$, respectively; not significant vs NG). A nonsignificant tendency to lower the expression of adhesion molecules was seen with 10 ng of ruboxistaurin.

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Abbreviations: (DAG) diacylglycerol, (DMEM) Dulbecco's modified Eagle's medium, (HG) hyperglycemic, (HUVEC) human umbilical vein endothelial cells, (ICAM-1) intercellular adhesion molecule-1, (NG) normoglycemic, (NO) nitric oxide, (PBS) phosphate-buffered saline, (PC) personal computer, (PKC) protein kinase C, (PKC-2) protein kinase C-2, (RLU) relative light units, (VCAM-1) vascular cell adhesion molecule-1

Keywords: adhesion molecules, endothelial dysfunction, macrophage binding, protein kinase C, ruboxistaurin

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Abstract (continued)

Conclusions:

We conclude that monocyte adhesion to endothelial cells under hyperglycemic conditions is at least mediated by PKC β activation. Ruboxistaurin is able to suppress this monocyte adhesion even in a PKC β -specific concentration. Further studies should evaluate these potential effects of ruboxistaurin *in vivo*.

J Diabetes Sci Technol 2007;1(6):929-935