Effects of estrogens on endothelial-derived factors implicated in the atherothrombotic plaque vulnerability: Clarification of the molecular mechanisms.

N. Nasiri-Ansari 1, E. Spilioti 1, V. Kalotychou 1, K. Dalhman-Wright 2, P. Moutsatsou 1, A. Papavassiliou 1, E. Kassi 1

1 Laboratory of Biological Chemistry, Medical School, University of Athens, Greece
2 Department of Biosciences and Nutrition, Karolinska Institutet, Sweden

INTRODUCTION

Results from controlled perspective and randomized studies in postmenopausal women suggest that after the installation of atheromatosis and in the presence of atherosclerotic plaque, HRT may be potentially harmful. Among the key vessel wall component in the later stages of atherosclerotic process is endothelium. During the stages of plaque rupture and/or erosion, metalloproteinases (MMPs) and their inhibitors (TIMPs) seem to play a critical role. The plaque calcification implicates the RANKL/RANK/OPG molecules, also expressed in endothelial cells (ECs). Lysyl oxidase (LOX) expression was been recently inversely correlated with plaque instability. Inhibition of monocyte chemoattractant protein-1 (MCP-1) has been postulated to be a direct mediator of plaque instability. ADAMTS-4, a member of metalloproteinase family, found to cleave extracellular matrix components and has emerged as potential pathogenic factor of plaque instability. Finally, plaque instability may be mediated by the formation of new microvessels, and decreased vessel stability resulting from decreased PDGF (platelet derived growth factor).

Herein, we aimed 1) to investigate the effect of estrogens on the regulation of OPG-RANKL-RANK system, MMP-2 and MMP-9 and its inhibitors TIMP-1 and TIMP-2, ADAMTS-4, MCP-1, LOX, PDGF, in endothelial cells, 2) to clarify the molecular mechanism mediating these effects (through ERa, ERβ or GPR30).

METHODS

- We investigated the gene expression of ERa, ERβ and GPR30 in HAECS, at the mRNA level (real-time PCR) and the corresponding protein level expression (western blot analysis).
- HAECS incubated with estriadiol for 6, 12, 24 hours at concentrations 10^{-7}, 10^{-9}M in the absence of TNF-α, or preincubated with TNF-α (2ng/ml) for 24 hours (resembling low-grade inflammation state of established atherosclerosis).
- Real Time-PCR was used to qualitatively detect gene expression of MMP-2, MMP-9, TIMP-1, TIMP-2, OPG, RANKL, RANK, MCP-1, LOX, PDGF and ADAMTS-4.
- Gelatin zymography was used to detect MMP-2, MMP-9 activities in culture supernatants.
- In order to clarify the role of the nuclear receptors ERa and ERβ in the estradiol- mediated effects, we transfected HAECS with expression plasmids for either ERa or ERβ and repeated incubation with estradiol (10^{-7} - 10^{-9}M).

RESULTS

- Incubation of HAECS with estradiol at the lower concentration (1.1nM) for 24 hours increased the expression of LOX and MCP-1 gene, while does not affect the expression of MMP-2 and MMP-9 and its inhibitors TIMP-1 and TIMP-2, ADAMTS-4, PDGF, OPG, RANK genes. Since the only ER subtype expressed by HAECS at protein level was GPR-30, the above effects are mediated through this ER-subtype.
- Western blot analysis revealed the presence of GPR30 as the only ER subtype in HAECS (SK-BR-3 cells were used as positive control).
- In ERα-transfected HAECS, incubation with estradiol led to upregulation of TIMP-1 and TIMP-2 and in marginal increase of LOX and MCP-1 gene.
- In ERβ-transfected HAECS estradiol increased the expression of MCP-1 and LOX while decreased the expression of PDGF gene.

Conclusions

Estradiol regulates differently the expression of various genes implicated (either protectively or harmfully) in the atherosclerotic plaque instability depending on the ER subtype, with ERα and GPR-30 exerting more favorable effects at lower concentrations. In the presence of TNFα (resembling the low grade inflammation state of established atherosclerosis) the effect of estradiol appears to be more harmful as reflected by the highly increased MCP-1 and MMP-9 expression. The balance of expression of the ER subtypes (ERα, ERβ and GPR-30) seems to play important role in the paradoxical characterization of estrogens as both beneficial and harmful.

References