Role of Caveolin-1 in EGCG-Mediated Protection Against Linoleic Acid-Induced Endothelial Cell Activation

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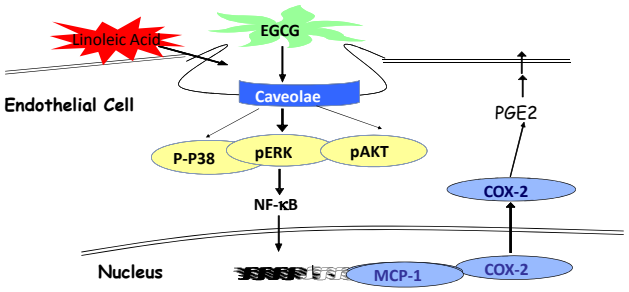
Abstract:

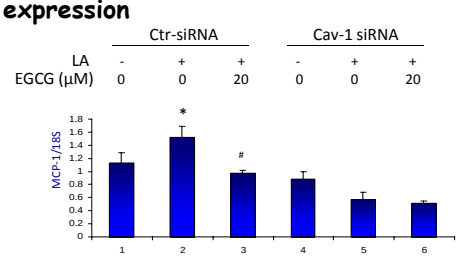
Flavonoids can protect against inflammatory diseases such as atherosclerosis by decreasing vascular endothelial cell activation. Plasma microdomains called caveolae are abundant in endothelial cells and play a major role in regulating signaling pathways associated with the pathology of vascular diseases. We hypothesize that flavonoids are antiinflammatory by modulating caveolae-regulated cell signaling. We focused on the role of caveolae and its major protein, caveolin-1, in mechanisms of linoleic acid-induced endothelial cell activation and protection by the green tea epigallocatechin gallate (EGCG). Pretreatment with EGCG blocked fatty acid-induced caveolin-1, MCP-1 and COX-2 expression. Similar results were observed with NF-κB DNA binding activity. Caveolin-1 silencing blocked linoleic acid-induced expression of MCP-1 and COX-2. Exposure to linoleic acid rapidly increased phosphorylation of several kinases, including p38 MAPK, ERK, and Akt. Inhibitors of ERK and Akt down-regulated the linoleic acid-induced increase in COX-2 protein. Our data provide evidence that caveolae may play a critical role in regulating vascular endothelial cell activation and protection by flavonoids such as EGCG.

Introduction:



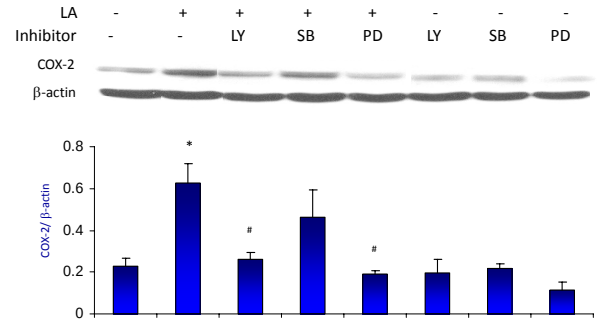
Results:

Caveolin-1 silencing blocked linoleic acid induced MCP-1 RNA expression



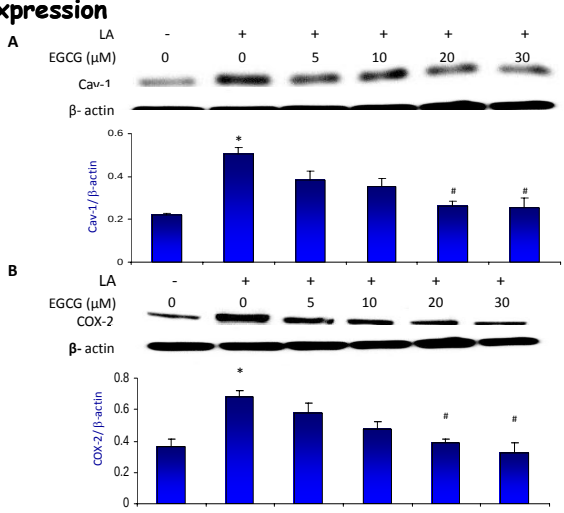
Caveolin-1 silencing reduces linoleic acid-induced activation of MCP-1. Endothelial cells were transfected with siRNA for caveolin-1 (Cav-1 siRNA) or with control siRNA (Ctr- siRNA) and treated with EGCG, followed by exposure to linoleic acid. Cav-1, MCP-1 and 18S RNA expression were determined by real-time PCR. *Significantly different compared to control cultures. #Significantly different compared to cultures treated only with LA.

The Akt and ERK1/2 pathways are involved in linoleic acid-mediated COX-2 expression



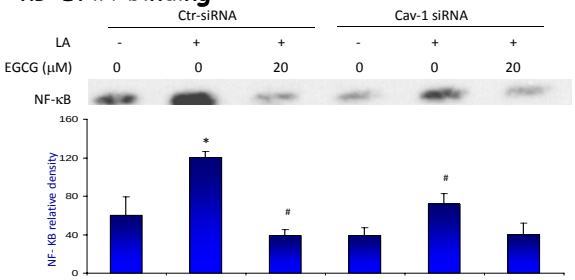
The Akt and ERK1/2 pathways are involved in linoleic acid-mediated COX-2 activation. Endothelial cells were pretreated with or without inhibitors to Akt (LY294002), p38 MAPK (SB 203580), or ERK (PD98059), followed by exposure to linoleic acid (LA). Activation of COX-2 was determined by western blot analysis. *Significantly different compared to control cultures. #Significantly different compared to cultures treated only with LA.

EGCG blocks linoleic acid-induced COX-2 and caveolin-1 expression



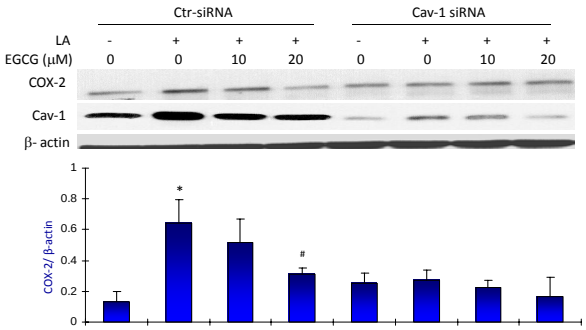
EGCG blocks linoleic acid-induced COX-2 and caveolin-1 expression in a concentration-dependent manner. Cells were pretreated with either vehicle or EGCG, followed by exposure to linoleic acid (LA). Caveolin-1 (Figure A) and COX-2 (Figure B) protein activation were determined by western blot analysis. *Significantly different compared to vehicle control. #Significantly different compared to cultures treated only with LA.

Caveolin-1 silencing reduces linoleic acid-induced NF-κB DNA binding



EGCG and caveolin-1 silencing can both reduce linoleic acid induced NF-κB DNA binding. Endothelial cells were transfected with siRNA for caveolin-1 or with control siRNA and treated with EGCG, followed by exposure to linoleic acid. Electrophoretic mobility shift assay for NF-κB was performed with nuclear proteins extracted from endothelial cells. *Significantly different compared to vehicle control. #Significantly different compared to cultures treated only with LA.

Caveolin-1 silencing reduces linoleic acid-induced expression of COX-2



Caveolin-1 silencing reduces linoleic acid-induced activation of COX-2. After transfection, endothelial cells were treated with EGCG, followed by exposure to linoleic acid. Cell lysates were probed with Cav-1, COX-2 and β-actin antibodies. Protein expression of caveolin-1 and COX-2 was determined by western blot analysis. *Significantly different compared to control cultures. #Significantly different compared to cultures treated only with LA.

Acknowledgements:

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Conclusions:

- Caveolin-1 silencing blocked linoleic acid-induced endothelial activation and inflammation.
- EGCG decreased fatty acid-induced NF-κB DNA binding activity and MCP-1 and COX-2 expression.
- Pretreatment with EGCG blocked fatty acid-induced caveolin-1 protein expression, suggesting that protective properties of EGCG may be caveolae-dependent.