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Interference of Advanced Glycation End-products signaling with collagen cross-linking in human endothelium

Christos Adamopoulos[1], Christina Piperi[1], Penelope Korkolopoulou[2], Georgia Dalagiorgou[1], Anastasia Spyropoulou[1], Antonios N. Gargalionis[1], Eleni A. Kandaraki[1], Athanasios G. Papavassiliou[1]

National & Kapodistrian University of Athens

[1]*Department of Biological Chemistry,* [2]*First Department of Pathology, Medical School, University of Athens, Athens, Greece*

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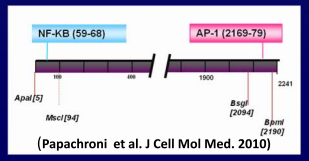
Introduction:

Maintenance of extracellular matrix (ECM) stability is critical for vascular remodeling associated with cardiovascular diseases. Covalent cross-linking of collagen and elastin initiated by the copper-dependent lysyl oxidase (LOX) is a central event assuring ECM stability and vascular homeostasis. LOX downregulation leads to endothelial dysfunction characteristic of early atherosclerotic stages, whereas its upregulation in vascular cells can induce neointimal thickening in atherosclerosis and restenosis. Advanced Glycation End-products (AGEs), the highly reactive products of non-enzymatic glycation of proteins, lipids and nucleic acids, contribute to endothelial dysfunction, atherosclerosis and vascular injury under both normal and diabetic conditions.

Aim:

The aim of the present study was to investigate the effect of AGEs in regulation of LOX gene/protein expression in human endothelial cells and to explore the potential functional impact of this interaction in an animal model.



(Papachroni et al. J Cell Mol Med. 2010)

LOX gene promoter has binding sites for AGE-induced transcription factors **NF-κB** and **AP-1** transcription factors

Methods & Materials:

• Cell Cultures: Human Aortic Endothelial Cells (HAECs)

• AGE-Bovine Serum Albumin (AGE-BSA)-treatment
Concentrations: 100, 200 μg/ml - Time points: 24, 48, 72h

• Quantitative real-time Polymerase Chain Reaction (real-time qPCR):
LOX mRNA expression

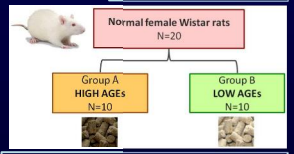
• Flow cytometry analysis:
RAGE and LOX protein expression

• Western Blot analysis:
activated/phosphorylated ERK1/2 (p-ERK1/2) expression

• Electrophoretic-Mobility Shift Assay (EMSA):
NF-κB and AP-1 binding to *LOX* gene promoter

• Immunohistochemistry:
AGEs, RAGE, LOX expression in normal aortic endothelium of rats fed with low- or high-AGE content diet.

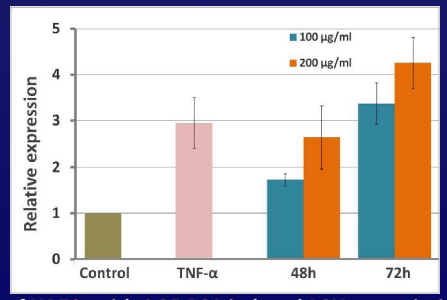
Phase contrast image HAECs



HIGH AGEs diet was prepared through exposure in 191° C for 30 min

Results:

I. *LOX* gene expression analysis (mRNA levels) in HAECs after treatment with AGEs

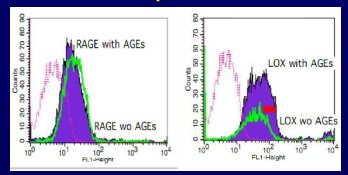


- total RNA extraction
- reverse transcription (RT-PCR)
- real time qPCR

TNF-α: positive control

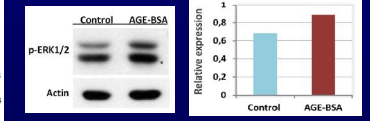
Treatment of HAECs with AGE-BSA induced *LOX* transcription in a time- and dose- dependent manner.

II. Flow cytometric analysis of RAGE & *LOX* expression in HAECs



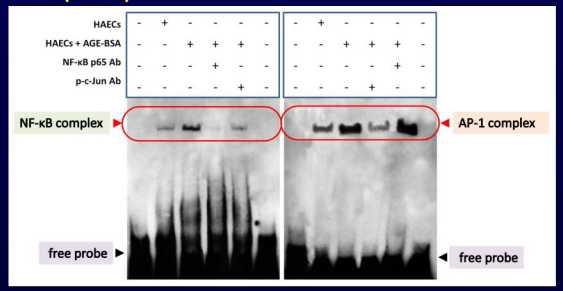
RAGE expression in HAECs remained unchanged while LOX expression increased after AGE-BSA treatment (72h, 100μg/ml).

III. Western blot for p-ERK1/2 in untreated (Control) and AGE-treated HAEC protein extracts



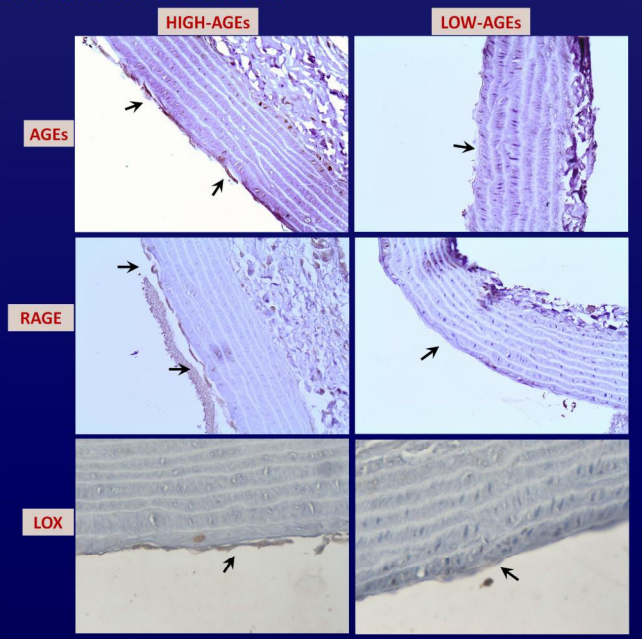
Induction of p-ERK1/2 expression after AGEs administration (72h, 100μg/ml).

IV. Analysis of *LOX* gene promoter binding capacity of transcription factors NF-κB and AP-1 (EMSA)



Treatment of HAECs with AGEs (72h, 100μg/ml) induced NF-κB and AP-1 activation and binding to *LOX* gene promoter.

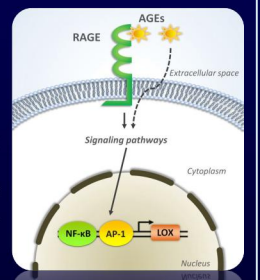
V. Immunohistochemical investigation of AGEs, RAGE and LOX in normal rat aortic endothelium



Increased expression and co-localization of AGEs, RAGE and LOX were observed in the aortic endothelium of normal rats fed with high-AGE diet compared with controls.

Conclusion

AGE-RAGE signaling induces **LOX** protein expression in **endothelium** through regulation of *LOX* gene promoter by the transcription factors, **NF-κB** and **AP-1** constituting a **molecular mechanism** that potentially contributes to the characteristic **endothelial dysfunction** of **obesity, diabetic microvascular complications, atherogenesis** and **polycystic ovary syndrome.**



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*chr.adamopoulos@gmail.com

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