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

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Introduction:
Maintenance of extracellular matrix (ECM) stability is critical for vascular remodeling associated with cardiovascular diseases. Cova lent cross-linking of collagen and elastin initiated by the copper-dependent lysyl oxidase (LOX) is a central event ensuring 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.

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

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

![LOX gene promoter has binding sites for AGE-induced transcription factors NF-κB and AP-1 transcription factors](image1)

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

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

![RAGE expression in HAECs and LOX expression in HAECs](image2)

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

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

![Western blot for p-ERK1/2 in untreated (Control) and AGE-treated HAEC protein extracts](image3)

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.

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

![EMSA of LOX gene promoter binding capacity of transcription factors NF-κB and AP-1](image4)

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

![Immunohistochemical investigation of AGEs, RAGE and LOX in normal rat aortic endothelium](image5)

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, atherosclerosis and polyvascular oamy syndrome.

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