MON-161

Transcriptional regulation of Endothelin-1 expression by Advanced Glycation End-products in human aortic endothelium is mediated via NF-kappaB and AP-1

Christos Adamopoulos[1*], Christina Piperi[1], Georgia Dalagiorgou[1], Antonios N. Gargalionis[1], Eleni A. Kandaraki[1], Evanthia Diamanti-Kandarakis[2], Athanasios G. Papavassiliou[1]

[1]Department of Biological Chemistry, Medical School, University of Athens - [2]Endocrine Unit, Third Department of Internal Medicine, Medical School, University of Athens, Athens, Greece

National & Kapodistrian University of Athens

HOUSTON ENDO 2012 — JUNE 23–26 — THE ENDOCRINE SOCIETY'S 94TH ANNUAL MEETING & EXPO

Introduction:

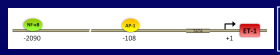
• **Advanced Glycation End-products** (**AGEs**) are produced by the non-enzymatic glycation of proteins, lipids and nucleic acids, resulting in an overload of highly reactive molecules of endogenous or exogenous (dietary) origin.

• Increased AGE levels in circulation and concomitant elevated tissue deposition have been associated with diabetic complications, atheromatosis, ageing and more recently with polycystic ovary syndrome pathogenesis.

• Interaction of AGEs with their receptor **RAGE** (**Receptor for AGEs**) activates intracellular signaling pathways which induce targeted gene expression in endothelium including upregulation of cell adhesion molecules and **endothelin-1** (**ET-1**), implicated in vascular injury and endothelial dysfunction.

Aim:

To explore the molecular mechanism of AGE-induced regulation of *ET-1* gene/protein expression in human endothelial cells and investigate its functional relevance in normal rat vascular endothelium.



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

Methods & Materials:

• Cell Cultures: **Human Aortic Endothelial Cells** (**HAECs**)

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

Phase contrast image HAECs

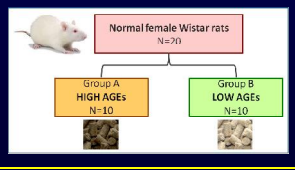
• Quantitative real-time Polymerase Chain Reaction (real-time qPCR): ET-1 mRNA expression

• Flow cytometry analysis: RAGE expression

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

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

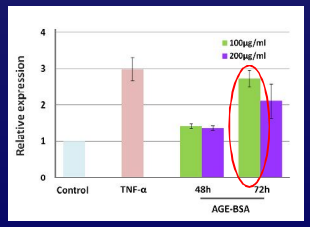
• Immunohistochemistry: AGEs, RAGE, ET-1 expression in normal aortic endothelium of rats fed with low- or high-AGE content diet.



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

Results:

I. *ET-1* gene expression analysis (mRNA levels) in HAECs after treatment with AGEs



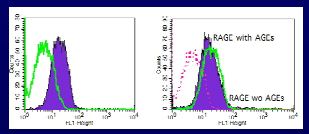
• total RNA extraction
• reverse transcription (RT-PCR)
• real time qPCR

Forward Primer	CCAAGGAGCTCCAGAAACAG
Reverse Primer	-GATGTCCAGGTGGCAGAAGT-

TNF-α: positive control

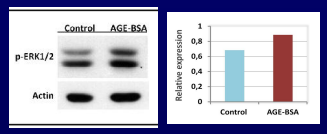
Treatment of HAECs with AGE-BSA induced *ET-1* transcription in a time- and dose- dependent manner.

II. Flow cytometric analysis of RAGE expression in HAECs



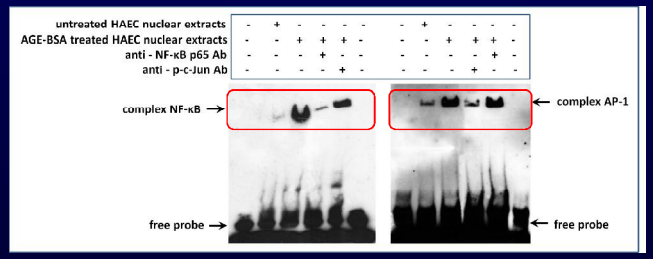
RAGE expression in HAECs remained unchanged after AGE-BSA treatment (72h, 100μg/ml).

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



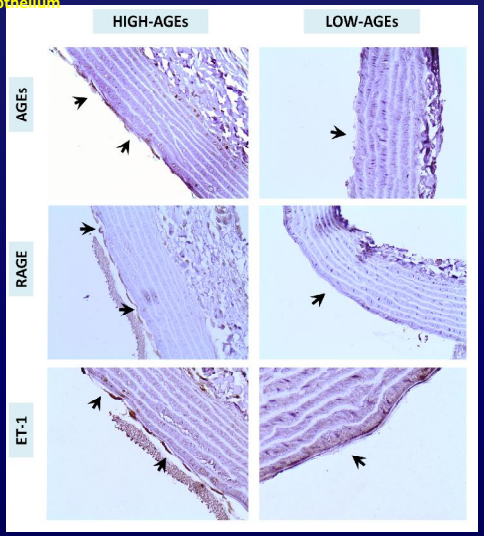
Induction of p-ERK1/2 expression after AGE-BSA administration (72h, 100μg/ml).

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



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

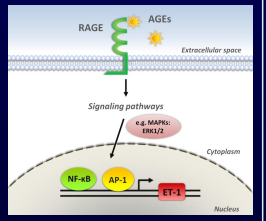
V. Immunohistochemical investigation of AGEs, RAGE and ET-1 in normal rat aortic endothelium



Increased expression and co-localization of AGEs, RAGE and ET-1 were observed in the aortic endothelium of normal rats fed with high-AGE diet compared with controls.

Conclusion

AGE-RAGE signaling induces **ET-1** protein expression in **endothelium** through regulation of *ET-1* 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.**



References:

Diamanti-Kandarakis E et al., Accumulation of dietary glycotoxins in the reproductive system of normal female rats. 2007 J Mol Med 85:1413-1420

Quehenberger P et al., Transcription Is Controlled by Nuclear Factor-κB in AGE-Stimulated Cultured Endothelial Cells. 2000 Diabetes 49:1561-1570

Christakou C et al., Strong and positive association of Endothelin-1 with AGEs in PCOS: A causal relationship or a bystander? 2011 Hormones 10:292-297

Deane R et al., RAGE mediates amyloid-β peptide transport across the blood-brain barrier and accumulation in brain. 2003 Nat Med. 9:907-913

*chr.adamopoulos@gmail.com

European Union — European Social Fund — Co- financed by Greece and the European Union
OPERATIONAL PROGRAMME EDUCATION AND LIFELONG LEARNING — investing in knowledge society — MANAGING AUTHORITY
NSRF 2007-2013

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.