A Study of the Vascular Basis of Alzheimer's Disease: The Role of Beta Amyloid (Aβ) Proteins and Saturated Fatty Acids in Endothelial Dysfunction and Inflammation

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Abstract

Background: Studies have shown that Alzheimer's Disease (AD) is strongly associated with the presence of atherosclerosis risk factors. We tested the hypotheses that β- amyloid proteins (Aβ) or palmitic acid (PA), a saturated fatty acid and known atherosclerotic risk factor, cause impaired function and viability of human umbilical vein endothelial cells (HUVEC), and that together, they exert synergistic effects on HUVEC dysfunction.

Methods: HUVECs were exposed for 18-20 hours to vehicle, Aβ42 (2μM) ± PA (150μM) or PA (150μM) while some HUVEC were exposed to a 4-hour pre-treatment with PA (150mM) followed wash and treatment with vehicle ± Aβ42 (2μM) for 18-20 hours. Outcomes measured included: (1) nitric oxide (NO) and measures of oxidative stress and nitrosative stress, (2) inflammatory and associated markers.

Results: HUVECs exposed to either Aβ or PA showed impaired NO production and increased superoxide and peroxynitrite when compared to vehicle control. Co-treatment with Aβ and PA did not cause a statistically significant change compared to Aβ or PA alone. HUVECs demonstrated variable inflammatory responses following exposure to either Aβ or PA.

Conclusion: Independent exposure of HUVECs to Aβ and PA caused decreased nitric oxide production and increased oxidative and nitrosative stress. HUVECs did not demonstrate Aβ-induced endothelial cell inflammation. Co-treatment with Aβ and PA did not result in a synergistic or additive increase in endothelial cell inflammatory responses.

Introduction

Alzheimer's disease (AD) is a leading cause of cognitive impairment that is expected to afflict 80 million people by year 2040. Both AD and stroke have been implicated as a cause of cognitive decline in elderly patients and, recent evidence indicates vascular risk factors contribute to the pathogenesis of both AD and stroke. There exists overwhelming evidence that the risk of developing AD is directly related to vascular risk factors including hyperlipidemia, hypternsion and diabetes.. However, mechanisms linking these risk factors to AD are not well understood; and the mechanisms by which they modulate Aβ-induced vascular dysfunction are not well established.

Methods

- HUVEC cells were exposed to vehicle, Aβ42 (2μM) ± PA (150μM), PA (150μM) for 18-20 hours or HUVECs were exposed to a 4-hour pre-treatment with PA (150mM) followed by treatment ± Aβ42 (2μM) for 18-20 hours.
- To determine endothelial function, nitric oxide was measured by NO analyzer, oxidative stress (superoxide) and nitrosative stress (peroxynitrite) were measured by flow cytometry.
- To measure HUVEC cell viability, markers of inflammation and matrix degradation, we measured interleukins (IL)-6, IL-8, Reaction for Advanced Glyocolytic End Products (RAGE) 1 and 2, and Matrix Metalloproteinases (MMP-9) by real-time PCR

Results

(a) HUVEC endothelial function

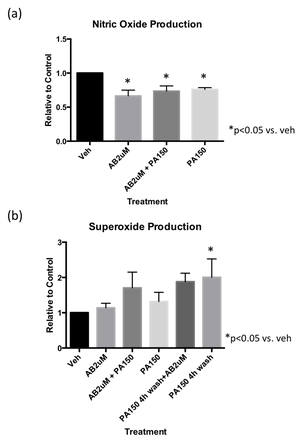

Figure 1. (a) HUVEC nitric oxide production. HUVECs exposed to Aβ42, PA, and co-treatment with both Aβ42 and PA demonstrated statistically significant decreases in nitric oxide production as compared to vehicle control (p=0.01, p<0, p<0.001, and p=0.01 respectively). The decrease in NO production for cells exposed to co-treatment did not result in a significant decrease when compared to the separate effects of Aβ42 and PA.

(b) HUVEC superoxide production. Sequential treatment of endothelial cells with PA for a 4-hour pre-treatment interval and treated for 18-20h with Aβ42 resulted in an 88% increase in superoxide production versus vehicle (p=0.03).

(c) Total peroxynitrite production as measured by flow cytometry. HUVECs exposed to treatment with Aβ42 (2μM), peroxynitrite production increased by 34% as compared to vehicle (p=0.03).

Results

(b) HUVEC cell viability, markers of inflammation and matrix degradation

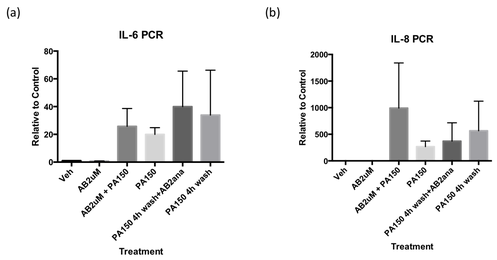

Figure 2. (a) HUVEC IL-6 gene expression as measured by rtPCR. Amplification of IL-6 gene expression was observed for all treatment modalities with the exception of Aβ42 (2μM), which resulted in a modest decrease in gene expression (b) Endothelial cell IL-8 gene expression. Similar to the results observed for IL-6, there was a non-significant upregulation of IL-8 gene expression for all treatment groups except for the HUVECs exposed to the Aβ42 treatment.

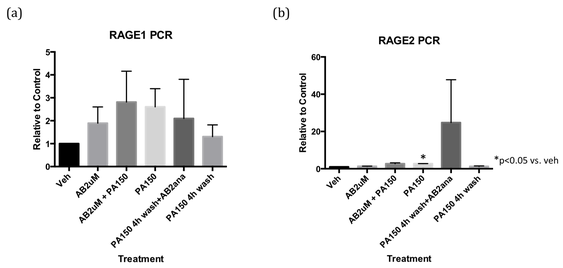

Figure 3. (a) HUVEC RAGE1 gene expression. In general, amplification of RAGE1 was upregulated by all treatment modalities. (b) RAGE2 gene expression. Endothelial cells exposed to treatment with PA resulted in a statistically significant increase in RAGE2 gene expression versus vehicle (p<0.003).

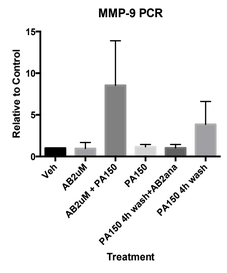

Figure 4. HUVEC MMP-9 gene expression as measured by rtPCR. HUVECs exposed to PA showed greater degree of amplification of MMP-9 from control which did not reach statistical significance.

Discussion

We present the following findings: 1) Exposure to Aβ42 and palmitic acid leads to significant endothelial dysfunction that is associated with oxidative/nitrative stress and reduced endothelial cell NO production. Contrary to our hypothesis, with the doses used, there appears to be no additive or synergistic effects when AB42 or PA were co-treated. 2) While there is an observable trend in the upregulation of inflammatory pathways as measured by IL-6, IL-8, RAGE1, RAGE2 and MMP-9, the upregulation in gene expression did not reach statistical significance.

Conclusions

Atherosclerosis and Alzheimer's disease, independently, comprise two of the largest public health burdens in aging and elderly populations. Current evidence including clinical, epidemiological and experimental data indicates that together, they appear to act in an additive or potentially synergistic fashion in the progression of AD. Our study attempted to address the lack of understanding surrounding the interplay of atherosclerosis risk factors and amyloid beta protein deposition on the development and progression of AD.

The data presented are consistent with, but do not prove, the original hypothesis that Aβ and palmitic acid both independently induce endothelial dysfunction through reduced nitric oxide production and pro-inflammatory cytokine production.

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