Protein-associated Chemical Switches Differentially Modulate the Binding Activities of Human Apolipoprotein E Isoforms with Their Receptors and with Amyloid Beta Peptides.

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INTRODUCTION

Apolipoprotein E (ApoE) acts as a major cholesterol carrier supporting lipid transport and tissue repair in the brain as well as mediates clearance of amyloid-beta peptides. ApoE proteins bind to several cell surface receptors including low-density lipoprotein receptor (LDLR) and very low-density lipoprotein receptor (VLDLR) to deliver lipids. ApoE also binds to Sortilin which mediates uptake of ApoE containing lipoproteins into neurons, and to amyloid- beta (Aβ) peptide, which is thought to play important roles in the pathogenesis of Alzheimer Disease (AD).

The ApoE gene is the main genetic determinant of AD risk in humans. The ApoE gene is polymorphic, having three prominent alleles, ApoE2, ApoE3, and ApoE4, with ApoE3 being the most common allele. ApoE4 carriers are significantly more likely to develop AD, while ApoE2 carriers have a moderately reduced risk of AD.

We recently reported that naturally occurring physiological chemicals (such as bicarbonate and sodium hydrogen sulfide) modulate the binding activities of antibodies as a function of the differential external cellular pH between the acidic tumor microenvironment (pH 5.8-6.7 resulting from glycolysis) and the alkaline environment of blood and normal tissues (pH 7.4 and higher) and is referred to as Protein-associated Chemical Switches or PaCS[1]. This discovery enabled the use of these physiological PaCS chemicals to increase the therapeutic index of cancer therapies. Since both senescent and other inflamed cells associated with AD are also glycolytic, leading to acidic external microenvironments, we investigated the effects of PaCS molecules on the binding activities of different ApoE isoforms to amyloid-β (Aβ) peptide and cell surface receptors as a function of pH.

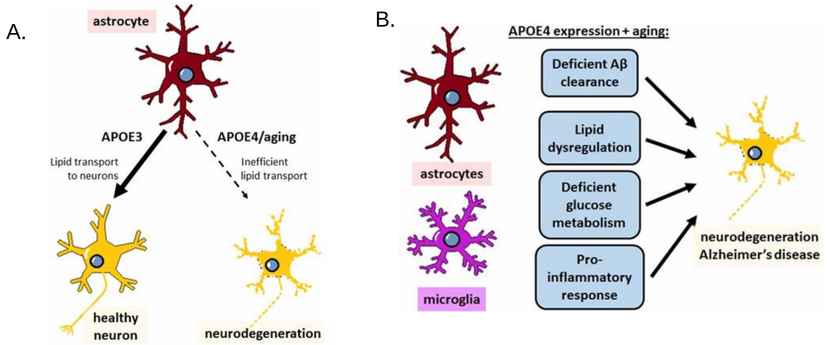

Figure 1: ApoE3 expression vs ApoE4 expression (A) Astrocytes expressing ApoE4 have inefficient lipid transport, sensitizing neurons to degeneration. (B) ApoE4 expression disrupts multiple homeostatic pathways in astrocytes and microglia to cause neurodegeneration and AD[2].

1. *Chang HW, Frey G, Liu H, Xing C, Steinman L, Boyle WJ, Short JM. Proc Natl Acad Sci U S A. 2021 Mar 2;118(9).*

2. *The Role of APOE4 in Disrupting the Homeostatic Functions of Astrocytes and Microglia in Aging and Alzheimer's Disease Celia G.Fernandez, et al. (2019). Frontiers in Aging Neuroscience 11 (14)*

RESULTS

Figure 2: Influence of the PaCS molecule, sodium bicarbonate, on the binding of ApoE Isoforms to amyloid beta peptides, LDLR, VLDLR and Sortilin at pH 6.0 and pH 7.4 is concentration dependent

- ApoE4 showed the highest binding activities at both acidic and alkaline conditions compared to other ApoE isoforms.
- Sodium bicarbonate affected the binding of ApoE isoforms to amyloid beta peptides, LDLR, VLDLR and Sortilin in a concentration dependent manner.
- Considerably reduced binding activities were observed under alkaline conditions vs acidic conditions in the presence of sodium bicarbonate for all ApoE isoforms.

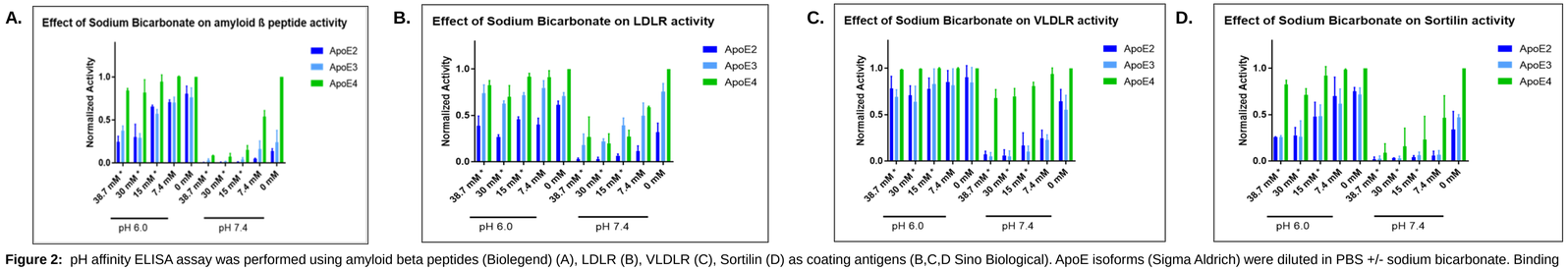

Figure 2: pH affinity ELISA assay was performed using amyloid beta peptides (Biolegend) (A), LDLR (B), VLDLR (C), Sortilin (D) as coating antigens (B,C,D Sino Biological). ApoE isoforms (Sigma Aldrich) were diluted in PBS +/- sodium bicarbonate. Binding of the ApoE isoforms to the antigens were detected with anti-ApoE antibody (Biolegend) and anti-mouse IgG conjugated with HRP (Promega). Bicarbonate physiological range are marked with an asterisk. Data were normalized to ApoE4 values.

Table 1: Influence of sodium bicarbonate on the pH selectivity of ApoE Isoforms binding to amyloid beta peptides, LDLR, VLDLR and Sortilin

ApoE isoforms	EC50-pH 6.0, PBS (ug/ml)	EC50-pH 7.4, PBS (ug/ml)	EC50-pH 6.0, PBS + sodium bicarb (ug/ml)	EC50-pH 7.4, PBS + sodium bicarb (ug/ml)	**Fold change - PBS (pH 6.0/pH 7.4)**	**Fold change - PBS + sodium bicarb (pH 6.0/pH 7.4)**	
ApoE2	1.48	5.43	4.64	26.72	**3.67**	**5.75**	Amyloid β peptides
ApoE3	2.19	9.05	4.68	28.64	**4.13**	**6.12**	
ApoE4	0.35	2.53	1.22	13.36	**7.23**	**10.91**	
ApoE2	2.14	7.33	5.98	16.68	**3.43**	**2.79**	LDLR
ApoE3	2.30	4.75	2.04	6.03	**2.06**	**2.96**	
ApoE4	0.60	3.05	1.52	6.56	**5.11**	**4.32**	
ApoE2	1.33	4.64	2.49	10.63	**3.49**	**4.26**	VLDLR
ApoE3	2.34	6.79	2.45	17.24	**2.90**	**7.05**	
ApoE4	0.41	1.08	0.48	3.10	**2.65**	**6.48**	
ApoE2	1.77	4.71	4.15	19.70	**2.66**	**4.75**	Sortilin
ApoE3	2.06	7.84	4.45	19.02	**3.80**	**4.27**	
ApoE4	0.38	2.10	1.36	7.38	**5.55**	**5.45**	

Table 1: pH affinity ELISA assay was performed using amyloid beta peptides, LDLR, VLDLR and Sortilin as coating antigens. ApoE isoforms were serially diluted in PBS without sodium bicarbonate (PBS) or in PBS with 30 mM sodium bicarbonate (PBS + sodium bicarb) at pH 6.0 or pH 7.4. Binding of the ApoE isoforms to the antigens were detected with anti-ApoE antibody and anti-mouse IgG conjugated with HRP. EC50 values were calculated using the nonlinear fit model (variable slope, four parameters) built into GraphPad Prism software version 7.03. EC50 (ug/mL) values were averaged from two representative experiments. Fold change were calculated using EC50 values of pH 7.4 divided by EC50 values of pH 6.0.

Observations:

- PaCS molecules generally reduced the binding of all ApoE isoforms against all four protein targets in both acidic and alkaline microenvironments, with the greatest reduction of binding occurring under alkaline conditions.
- The binding activities of ApoE4 to amyloid beta peptides, LDLR and Sortilin has the highest fold change between pH 6.0 and 7.4 compared to ApoE2 and ApoE3 in the presence of sodium bicarbonate.
- ApoE2 showed a greater decrease in binding activity to LDLR in alkaline conditions in the presence of sodium bicarbonate compared to ApoE3 and ApoE4.
- The binding activity of ApoE3 to LDLR was not affected by the presence of sodium bicarbonate in acidic conditions in contrast to ApoE2 and ApoE4.

CONCLUSIONS

Multiple factors are implicated for the manifestation of AD, including viral infection, chronic inflammation, senescent cells, poor protein recycling and many other underlying factors that are associated with the build up of amyloid containing plaques. Astrocytes, for example, which play an important role in amyloid beta clearance, can initiate senescence in response to certain stressors. Astrosenescence leads to the production of proinflammatory factors know as senescence-associated secretory phenotype (SASP) which are involved in the initiation and progression of AD and are known to be glycolytic.

In this study, we investigated the effects of PaCS molecules on the binding activities of different ApoE isoforms to amyloid-β (Aβ) peptide and cell surface receptors as a function of pH. At physiological concentrations, bicarbonate and sodium hydrogen sulfide (data not shown) were observed to differentially affect the binding activities of the ApoE isoforms to LDLR, VLDLR, Sortilin and Aβ peptide. We observed large reductions in binding of ApoE under alkaline conditions versus acidic conditions in the presence of PaCS molecules, with ApoE4 showing the highest binding activity, especially under acidic conditions relative to the other isoforms. In addition to the data presented here, we performed pH ELISA with conditions ranging between pH 6.0 to pH 7.4. Results indicated ApoE isoforms have higher binding activity at pH 6.5 compared to pH 7.4. We are sharing the pH 6.0 data to underscore the potential impact of PaCS. Our data indicates that PaCS molecules can conditionally (pH dependent) modulate the interactions of the different ApoE isoforms with various cell surface receptors. These results provide potential mechanistic insights into the differential activity of the ApoE isoforms in normal, alkaline blood versus acidic microenvironments associated with inflammation, as well as insights into the potential isoform-dependent protection from PaCS molecules in the pathogenesis of neurodegenerative diseases. These data suggest that PaCS-dependent Conditionally Active Biologic or CAB therapies that target proteins or cells in these glycolytic, acidic microenvironments in the brain may lead to safer and more potent therapies that might also be administered at earlier stages of AD progression for improved outcomes.

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