Differences in Carotenoid Content of Normal Elderly and Alzheimer's Brains

Neal E. Craft, Dennis Gierhart, and C. Kathleen Dorey

Craft Technologies, Inc., ZeaVison, LLC, Virginia Tech Carilion School of Medicine

Introduction

Alzheimer's Disease (AD) is a severe neurodegenerative disease. AD and other dementia afflicts >36 million elderly worldwide. Antioxidants, such as carotenoids, have been implicated in the prevention of degenerative diseases and recent data correlates blood and macular carotenoid levels with cognition

Methods

- Brain sections received frozen from Mass. General Hospital were dissected into gray and white matter.
- 1-3 g of tissue was extracted 3x with 90 hexane/10 ethyl acetate.
- A portion was saponified with KOH for 60 min in the presence of pyrogallol.
- The mix was diluted with water and extracted 3x with hexane/ethyl acetate.
- The combined extract was washed with water then evaporated under nitrogen.
- The residue was dissolved in 25 uL ethyl acetate and diluted with 75 uL mobile phase and centrifuged prior to injection.
- Data were analyzed by ANOVA. P-values <0.05 were considered significant.

Purpose:

- To identify and quantify the major Carotenoids, Tocopherols, and Retinol in elderly normal and AD human brain.
- To determine if analyte concentrations are different in normal vs AD brain.
- To determine if analyte concentrations are different in White vs Gray Matter.
- To determine if analyte concentrations are different in brain regions more vulnerable to AD.

HPLC Conditions:

- Column: Spherisorb ODS2, 3um, 250 x 4.0 mm with Ti frits
- Mobile Phase: 80 ACN/15 Dioxane/2.5 MeOH/2.5 IPA/0.1 TEA (150 mM AmOAc in alcohol components)
- Temp: 31°C
- Flow: 1.2 mL/min
- Detect: Visible 450 nm; Fluor 330nm Ex/460nm Em, 296nm Ex/340nm Em
- Calib: Ext. Std. using peak area

Results

Figure 1. Chromatogram of Carotenoid Separation

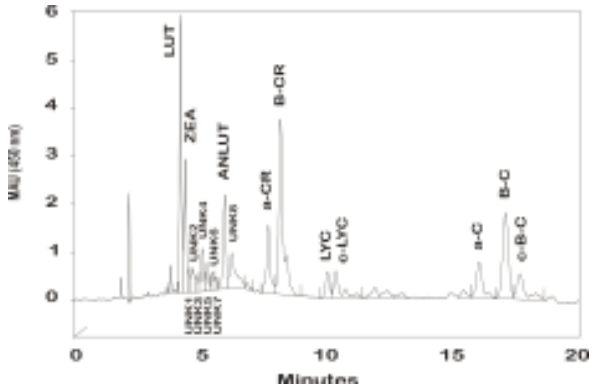

Table 1 Comparison of Analyte Concentrations in AD vs C Brain and Gray vs White Matter

	ANOVA p-value	
Analyte	**AD vs C**	**G vs W**
Lutein	**0.046**	**0.001**
Zeaxanthin	**0.001**	**0.010**
Anhydrolutein	0.057	0.774
a-Cryptoxanthin	0.505	0.885
B-Crytoxanthin	0.942	0.232
Lycopene	0.057	0.864
a-Carotene	0.262	0.733
B-Carotene	0.268	0.130
Total Carotenes	0.103	0.556
Retinol	**0.005**	0.710
a-Tocopherol	**0.005**	0.591
g-Tocopherol	0.374	0.352
d-Tocopherol	0.942	0.266
Total Tocopherols	**0.006**	0.695
Lutein + Zeaxanthin	**0.009**	**0.002**
Total Antioxidants	**0.006**	0.701

Brain tissue donated by Mass. Alzheimer's Disease Research Ctr. Funded in part by Applied Food Biotechnology and ZeaVision LLC.

Email: ncraft@crafttechnologies.com

Figure 2. Summary of Carotenoid and Retinol Levels in Control and Alzheimer's Brains

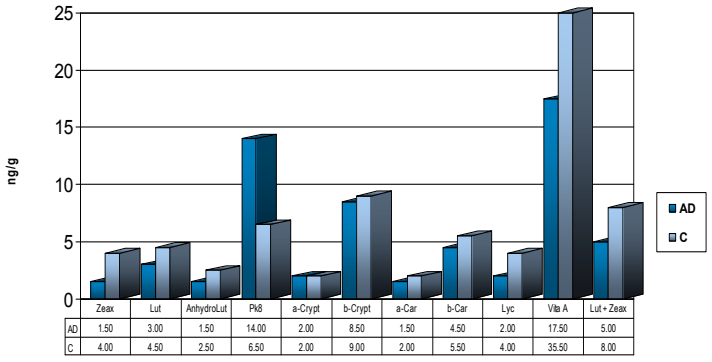

Table 2 Comparison of Unidentified Peaks in AD vs C Brain and Gray vs White Matter

	ANOVA P-value	
Analyte	**AD vs C**	**G vs W**
Peak 1	0.060	0.263
Peak 2	**0.026**	0.934
Peak 3	**0.046**	0.849
Peak 4	**0.005**	0.981
Peak 5	0.139	0.859
Peak 6	0.073	0.824
Peak 7	0.174	0.196
Peak 8	**0.003**	0.902

Conclusions

At least 16 carotenoids, 3 tocopherols, and retinol were present in human brain (Tables 1 and 2). Xanthophylls accounted for >70% of carotenoids in brain. Mean concentrations of carotenoids ranged from 3.5-15.3 pmol/g (Figure 2). Combining all brains, lutein (LUT, P<0.001) and zeaxanthin (ZX, P<0.01) were higher in gray vs white matter. ZX and LUT were lower in AD brain (P<0.001, P<0.046, respectively). Healthy brain had 2x more ZX and 30% more LUT than AD brain. Retinol and a-tocopherol were lower in AD brain, (p<0.005, P<0.005). A carotenoid peak, tentatively labeled peak 8, was positively correlated with AD and shares Lutein's UV/visible spectrum.