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DIETARY CHOLESTEROL INCREASES VENTRICULAR VOLUME AND NARROWS CEREBROVASCULAR DIAMETER IN A RABBIT MODEL OF ALZHEIMER'S DISEASE

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Abstract—Using structural magnetic resonance imaging in a clinical scanner at 3.0 T, we describe results showing that following 12 weeks on a diet of 2% cholesterol, rabbits experience a significant increase in the volume of the third ventricle compared to rabbits on a diet of 0% cholesterol. Using time-of-flight magnetic resonance angiography, we find cholesterol-fed rabbits also experience a decrease in the diameter of a number of cerebral blood vessels including the basilar, posterior communicating, and internal carotid arteries. Taken together, these data confirm that, despite the inability of dietary cholesterol to cross the blood–brain barrier, it does significantly enlarge ventricular volume and decrease cerebrovascular diameter in the rabbit – effects that are also seen in patients with Alzheimer's disease.

Key words: Alzheimer's disease, animal model, ventricle, MRI, cerebrovasculature, copper.

INTRODUCTION

Alzheimer's disease (AD) has significant effects on the brain including changes in volume, structure, vasculature, extracellular content, intracellular composition and alters functional properties including synaptic plasticity and cognition (Nestor et al., 2008; Ondrejcak et al., 2010; Tampellini and Gouras, 2010; de la Torre, 2012; Lim et al., 2013; Roher et al., 2012; Leung et al., 2013; Yarchoan et al., 2013). Animals including transgenic and cholesterol-fed mice, cholesterol-fed rats, transgenic and cholesterol-fed rabbits, aged dogs and primates have all been proposed as models of AD that recapitulate one or more aspects of this disease (Gotz and Ittner, 2008; Woodruff-Pak, 2008; Philipson et al., 2010).

Among the most consistent and conspicuous anatomical changes found in AD is an increase in ventricular volume (Nestor et al., 2008; Leung et al., 2013). Although a hallmark in humans, surprisingly little has been reported on changes in ventricular volume in animal models of AD. In one imaging study, Andjus and colleagues found that a trimethylin-treated rat model of AD appeared to have enlarged ventricles compared to sham-treated controls when imaged by magnetic resonance imaging (MRI) at 1.5 T (Andjus et al., 2009). In another study, Dror et al. (2010) observed ventricle enlargement in a thiamine-deficient rat model of neurodegeneration using a 7.0 T magnet. Xie et al. (2010) found that a transgenic mouse model of AD exhibited ventricular dilation compared to wild-type mice examined at 4.7 T. Interestingly, Chen et al. (2010) noted ventricular enlargement in normal mice as a function of age using a 7.0 T MRI scanner suggesting enlargement may simply be a function of age in this model (Fjell et al., 2009). A study by Ramesh et al. (2011) showed that intracisternal injection of beta amyloid into the brains of aged rabbits reduced cortical thickness and increased the thickness of the lateral ventricles when measured 45 days after treatment.

Another consistent change found in AD is an alteration of the cerebrovasculature including the narrowing of vessels and the deposition of beta amyloid known as cerebral amyloid angiopathy (Bell, 2012; Roher et al., 2012; Sagare et al., 2012). In fact, there is considerable evidence suggesting that neurovascular damage may antedate other signs of pathology including beta amyloid accumulation in the brain (de la Torre, 2012), and may even be the cause of poor clearance of beta amyloid from the brain (Deane et al., 2009). One important index of cerebrovascular change that is thought to be a major contributor to AD pathology is the narrowing of vessel diameter that can lead to reductions in cerebral blood flow and hypoperfusion (de la Torre, 2012; Roher et al., 2012).

Cholesterol is important for cell structure, repair and signaling, hormone production and bile acid synthesis but is also a well-known risk factor for cardiovascular disease (Sprecher et al., 2007; Reis et al., 2013) and has been associated with AD and cognitive impairment (Whitmer et al., 2005; Purnell et al., 2009; Tolppanen et al., 2012). We and others have shown significant effects of cholesterol on learning and memory (Sparks

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Abbreviations: AD, Alzheimer's disease; apoE, apolipoprotein E; MIP, maximum intensity projection; MPRAGE, magnetization prepared reduced angle gradient echo sequence; MRI, magnetic resonance imaging; TOF, time-of-flight.

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and Schreurs, 2003; Dufour et al., 2006; Darwish et al., 2010; Ya et al., 2012) and cholesterol has been correlated with cognitive impairment (Yaffe et al., 2002; Solomon et al., 2007; Reis et al., 2013). A large body of data now confirms a strong link between cardiovascular disease and cognitive impairment (Whitmer et al., 2005; Nash and Fillit, 2006; Grodstein, 2007).

In the present study, we used both semi-automated measurements of the lateral and third ventricles and manual measurements of the diameter of individual blood vessels visualized using time-of-flight (TOF) magnetic resonance angiography in the same rabbits to determine the effects of cholesterol on the brain. We found a significant change in third ventricle volume as a result of the high-cholesterol diet and report that several cerebral arteries including the basilar, the posterior communicating, and the internal carotid arteries were narrowed as a function of the cholesterol diet. The sizes of these effects are comparable to those seen in patients with AD (Nestor et al., 2008; Roher et al., 2012).

EXPERIMENTAL PROCEDURES

Animals

A total of 54 male New Zealand White rabbits (*Oryctolagus cuniculus*) 3–4 months of age and weighing approximately 2 kg upon arrival were housed individually, with access to one cup (128 grams) of Purina rabbit chow per day and *ad libitum* water, maintained on a 12-h-light/12-h-dark cycle and treated following United States National Institutes of Health guidelines in experiments approved by the West Virginia University Animal Care and Use Committee.

The rabbits received one of four possible treatment conditions in a 2×2 factorial design in which food (cholesterol vs. normal chow) and water (copper vs. distilled water) were manipulated. Rabbits received either high-fiber Purina 5326 chow (containing 21% fiber and 0% cholesterol) and distilled water ($n = 15$), Purina 5326 chow and copper in distilled water ($n = 13$), Purina 5326 chow plus 2% cholesterol (Test Diet, Richmond, IN) and distilled water ($n = 9$), or Purina 5326 chow plus 2% cholesterol and copper in distilled water ($n = 17$). Rabbits given copper received copper sulfate in their distilled drinking water with a final copper concentration of 0.12 ppm (0.12 mg/L). Rabbits were kept on their respective diets for 12 weeks. The high fiber diet was used to decrease the rate of cholesterol absorption and the consequent hepatotoxicity that develops with cholesterol feeding in rabbits (Song et al., 2000; Kainuma et al., 2006; Sparks et al., 2007).

MRI methods

Animals were anesthetized using 27.7 mg/kg ketamine and 5.7 mg/kg of xylazine injected subcutaneously 15 min before imaging. A Verio 3.0 T clinical scanner (Siemens Medical Solutions USA, Inc., Malvern, PA, USA) was used with the body coil used for radio-frequency transmission and the Siemens eight channel foot and ankle coil used for signal reception. The rabbit's head was supported on a Plexiglas table to position each rabbit in the same prone position. The rabbit's body was extended onto foam supports with a heated Delta Phase Thermal pad (Braintree Scientific, Braintree, MA, USA) under the torso to maintain a core temperature of 36–38 °C. Each rabbit MRI image data set was collected in less than 30 min.

The rabbits underwent 3 MRI scans: (1) a 3-plane localizer scan to check animal positioning and prescribe the axial slices, (2) an axial T1-weighted 3D magnetization prepared reduced angle gradient echo sequence (MPRAGE) for anatomical scanning with good gray/white matter differentiation and dark cerebrospinal fluid (CSF); (3) a three-dimensional (3D) TOF magnetic resonance angiography sequence to image vessels. All axial image sets covered from below the most caudal end of the cerebellum to a slice beyond the rostral end of the olfactory bulb.

The MRI image acquisition parameters were as follows. The MPRAGE images were used for the current ventricle volume analysis and were 1.2 mm in slice thickness. The MPRAGE images had an in-plane resolution of 0.176 mm $\times$ 0.176 mm with image acquisition parameters as follows: slices = 104; TR/TE/TI = 1570 ms/4.3 ms/900 ms, flip angle = 25 degrees, bandwidth = 130 Hz/pixel, number of acquisitions = 2. The 3D TOF images had spatial resolution of 0.3125 mm $\times$ 0.3125 mm $\times$ 1.0 mm. The image acquisition parameters were; flow compensation in the slice direction, 3D TOF – TR/TE = 25 ms/4.1 ms, flip angle = 25 degrees, bandwidth = 266 Hz/pixel, 1 acquisition. Maximum intensity projection (MIP) images were created from the axial TOF images. MIP views were calculated for the axial, sagittal, and coronal planes.

Image analysis

To make ventricular volume measurements, the following image processing steps were performed. The MRI image contrast was enhanced using imaging software (ImageJ 1.46r, NIH) to saturate the pixels and normalize all images of each rabbit in the dataset. The lateral ventricles and the third ventricle were located with reference to a rabbit brain atlas (Girgis and Shih-Chang, 1981; Shek et al., 1986). Using the editing function of 3D Slicer – an MRI three-dimensional reconstruction program (Gering et al., 2001), the ventricles were manually selected using intensity-based threshold painting. The lateral ventricles were fully selected. The third ventricle was selected from the anterior edge of the cerebellum through the cerebral cortex. The statistics module of 3D Slicer was used for quantification of ventricular volume. The module derives the volume from the number of pixels selected with the editing module multiplied by the image spacing and volume per pixel. These volumes were measured three times and averaged by an investigator blinded to the treatment condition of the rabbits.

The vessel diameters were measured on the source images following the full-width half-maximum signal method as previously described (Lemieux et al., 2010) with the following modifications. The axial TOF source images were loaded into ImageJ (NIH) imaging