ELSEVIER

Neurobiology of Aging xxx (2009) xxx–xxx

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Anti-inflammatory drugs reduce age-related decreases in brain volume in cognitively normal older adults

K. Walther [a], B.B. Bendlin [b,c], E.L. Glisky [a], T.P. Trouard [d], J.R. Lisse [e], J.O. Posever [f], L. Ryan [a,*]

[a] *Cognition and Neuroimaging Laboratories, Department of Psychology, University of Arizona, Tucson, AZ, 85721, USA*
[b] *Department of Medicine, University of Wisconsin Medical School, Madison, WI, 53705, USA*
[c] *Geriatric Research Education and Clinical Center, William S. Middleton Memorial Veterans Hospital, Madison, WI, 53705, USA*
[d] *Biomedical Engineering Program, University of Arizona, Tucson, AZ, 85721, USA*
[e] *Arizona Arthritis Center, College of Medicine, University of Arizona, Tucson, AZ, 85724, USA*
[f] *Phoenix Indian Medical Center, Phoenix, AZ, 85016, USA*

Received 6 October 2008; received in revised form 20 January 2009; accepted 14 March 2009

Abstract

Previous studies have indicated a decreased risk for developing Alzheimer's disease in anti-inflammatory (AI) drug users. Yet few studies have determined whether AI drug use provides a protective effect against normal age-related changes in the brains of older adults. Regional volume changes in gray and white matter were assessed cross-sectionally using optimized voxel-based morphometry in 36 females taking AI drugs as arthritis or pain medication and 36 age- and education-matched female controls. Although mean gray and white matter volume differences between AI drug users and the non-AI group were small, AI drug use interacted with age, such that the non-AI group showed significantly greater age-related volume changes in regions of both gray and white matter compared to the AI drug users. These regions included the superior and medial frontal gyri, middle and inferior temporal gyri, fusiform and parahippocampal gyri, and occipital gray matter as well as temporal, parietal, and midbrain white matter. The results are consistent with the notion that AI drugs provide protection against age-related changes in brain volume. It is possible that inflammation plays a role in volume decreases associated with normal aging, and that suppressing the inflammatory response moderates this decrease.
© 2009 Elsevier Inc. All rights reserved.

Keywords: Aging; Alzheimer's disease; Voxel-based morphometry; Magnetic resonance imaging; Anti-inflammatory agents

1. Introduction

Early epidemiological studies have indicated that patients with arthritis are at decreased risk for developing Alzheimer's disease (AD; Breitner et al., 1995; McGeer et al., 1990), possibly due to the use of anti-inflammatory (AI) medications. It has been hypothesized that chronic inflammation of the brain is a central feature in the development of neuropathology associated with AD and that long-term AI medication use may suppress this inflammation, leading to decreased risk for development of the disorder (Aisen, 2002; Tuppo and Arias, 2005).

Reduced risk for AD has also been demonstrated in epidemiological studies focusing on non-steroidal anti-inflammatory drugs (NSAIDs), typically used as a treatment for arthritis. While some studies have demonstrated an inverse association between daily use of NSAIDs and AD (Breitner and Zandi, 2001; Broe et al., 2000; in't Veld et al., 2001; Szekely et al., 2008a; Vlad et al., 2008), other studies have failed to find a protective effect (Arvanitakis et al., 2008; Henderson et al., 1997; in't Veld et al., 1998). Supporting the notion of a protective effect of NSAIDs, several meta-analyses have revealed significant combined odds ratios between 0.49 and 0.72 (Etminan et al., 2003; McGeer et al., 1996; Szekely et al., 2004), suggesting up to a 50% decrease

* Corresponding author at: Cognition & Neuroimaging Laboratories, Depts. of Psychology and Neurology, University of Arizona, P.O. Box 210068, Tucson, AZ 85721-0068, USA. Tel.: +1 520 621 7443.
E-mail address: ryant@email.arizona.edu (L. Ryan).

0197-4580/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2009.03.006

Please cite this article in press as: Walther, K., et al., Anti-inflammatory drugs reduce age-related decreases in brain volume in cognitively normal older adults. Neurobiol. Aging (2009), doi:10.1016/j.neurobiolaging.2009.03.006

in risk for developing AD in NSAID users. The protective effect has been consistently stronger when NSAIDs were taken over longer periods of time (Etminan et al., 2003; in't Veld et al., 2001; Szekely et al., 2004; Vlad et al., 2008), at least 2 years or more (Breitner and Zandi, 2001).

In contrast to the overwhelmingly positive results of epidemiological studies, clinical trials of NSAIDs for the treatment of probable AD have yielded disappointing results (Aisen et al., 2002, 2003; Reines et al., 2004; Scharf et al., 1999). These studies suggested that NSAIDs may be useful prophylactically, rather than as a treatment for the disorder after the first symptoms appear. However, a large-scale primary prevention study that enrolled over 2500 participants (ADAPT Research Group, 2007) also yielded negative results, although the study included only individuals over the age of 70 years. Other studies have suggested that NSAIDs may not provide protection for AD when taken in the 2 years prior to dementia onset (Breitner and Zandi, 2001; in't Veld et al., 2001; Szekely et al., 2004). Collectively, these studies suggest that issues such as the age of the participants at commencement of drug use and the duration of use may be important factors, along with others, in assessing the putative beneficial effects of AI drugs.

Despite the considerable interest in this topic, surprisingly few studies have examined whether there is evidence for a protective effect of AI drug use in the brain in vivo. Bartolini et al. (2002) assessed rheumatoid arthritis patients taking AI drugs and found impaired cognition as well as hypoperfusion in frontal and parietal lobes as measured by SPECT, suggesting that AI drugs in rheumatoid arthritis patients might have a negative effect on brain functioning. However, the study was problematic in that it relied solely on normative data for comparison rather than including a carefully matched control group. Apart from this single study, there is virtually no information on how AI drugs might affect the function or structure of the brains of cognitively normal older adults. Finding a beneficial effect of AI drug use on age-related brain changes may provide an important first step in understanding the positive epidemiological results described earlier.

To address this issue, the present study employed voxel-based morphometry (VBM) in order to measure regional volumetric changes in gray and white matter in cognitively normal older adults taking AI medication and a group of age- and education-matched older adults not taking AI drugs. VBM is a semi-automated procedure for analysing high resolution magnetic resonance anatomical images that is capable of identifying regional volumetric differences associated with aging across the whole brain, applying statistics on a voxel-by-voxel basis rather than measuring volumes in a priori regions of interest (Ashburner and Friston, 2000; Mechelli et al., 2005). Based on the epidemiological evidence for a protective effect of AI medication against the development of AD, we hypothesized that AI drug use would result in preserved volumes of gray and white matter in older adults. In particular, we expect that AI drug use will be associated with preserved volumes in regions that are affected earliest in AD, including medial temporal lobe and parietal cortex. Studies have found that volume decreases in medial temporal lobe structures are already present in cognitively normal older adults with genetic risk for AD (Lemaitre et al., 2005; Wishart et al., 2006). Additionally, AI drug use may also show a protective effect on structures that are most strongly associated with normal aging, such as prefrontal cortex (Raz and Rodrigue, 2006).

2. Method

2.1. Participants

Seventy-two females (ages 52–92 years) living independently in the community were included in this study. All participants were screened to exclude a history of head injury with cognitive sequelae, neurological or psychiatric disorder, and past or present drug and/or alcohol abuse. Thirty-six females taking anti-inflammatory medications were recruited through the Arizona Arthritis Center at the University Medical Center in Tucson, Arizona. AI drugs were taken by participants as arthritis or pain medication for at least 2 continuous years at any time in the past, with a mean duration of 10.0 years. With the exception of two participants, all AI drug users were taking the medications at the time of the study. The two participants not on AI drugs at the time of the study had taken AI drugs for many years prior to the study and had stopped recently. AI drugs included non-selective NSAIDs, COX-2 inhibitor type NSAIDs, aspirin, and other drugs (see Table 1 for a complete description of the medications taken by participants). Some participants were also taking the disease modifying anti-rheumatic drug methotrexate, but all participants were taking other AI drugs. Aspirin was considered as an AI drug when the dosage exceeded 500 mg per day. Thirty-six females with no current or previous use of an AI drug other than for occasional headache (less than 12 times per year) were selected for the non-AI control group, and no non-AI control group participant had arthritis or other chronic pain condition that required medication. Non-AI individuals were recruited from the community through the use of newspaper advertisements and an existing database of older individuals who had participated in previous studies in our laboratory. They were matched to the AI drug user group on age and education (see Table 2). All pro-

Table 1
Number of participants taking AI drugs by drug type.

Drug type	Only	Methotrexate	Mix
Non-selective NSAIDs[a]	11	5	3
COX-2 NSAID[b]	2	2	4
Aspirin/zorprin	5	0	0
Other[c]	1	3	0

[a] Diclofenac, ibuprofen, meloxicam, naproxen, oxaprozin, piroxicam, salsalate, sulindac.
[b] Celecoxib, rofecoxib, valdecoxib.
[c] Infliximab/remicade, hydroxychloroquine, steroid, sulfasalazine.

Please cite this article in press as: Walther, K., et al., Anti-inflammatory drugs reduce age-related decreases in brain volume in cognitively normal older adults. Neurobiol. Aging (2009), doi:10.1016/j.neurobiolaging.2009.03.006

Table 2
Demographic information and neuropsychological test scores for the non-AI control group and AI drug users.

	Non-AI group ($n = 36$)			AI drug user ($n = 36$)		
	M	SD	Range	*M*	SD	Range
Age (years)	69.4	9.4	52–88	66.6	9.2	52–92
Education (years)	14.8	2.3	12–20	15.1	2.9	12–20
MMSE	28.9	1.3	24–30	29.2	1.0	27–30
WASI-vocabulary (raw score)	69.8	4.9	59–79	69.4	7.2	44–78
Memory function (*z*-score)	0.3	0.7	−1.9–1.3	0.5	0.5	−0.4–1.3
Executive function (*z*-score)	−0.1	0.6	−1.5–1.3	0.0	0.6	−1.6–1.6
Years of AI drug use				10.0	10.2	2–57

cedures were approved by the Human Subjects Committee of the University of Arizona, and written informed consent was obtained from each participant.

All participants underwent a detailed neuropsychological assessment that included the Mini Mental State Exam (MMSE; Folstein et al., 1975), the vocabulary subtest from the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999), and a series of tests measuring memory and executive functioning (described in detail in Glisky and Kong, 2008). All participants were functioning within the normal range for both memory and executive function based on Glisky and Kong's (2008) normative scores.

2.2. MRI imaging

Images were acquired on a GE 3.0T Signa VH/I whole body echospeed scanner (General Electric, Milwaukee WI). A set of 3-plane localizer images were followed by a 3D spoiled gradient-echo MRI (3DSPGR) scan used to obtain T1-weighted images of the whole brain with a section thickness of 0.7 mm (TR = 5.1 ms, TE = 2 ms, TI = 500 ms; flip angle = 15°; matrix = 256 × 256; FOV = 260 mm × 260 mm). Other scans were acquired at the same time but are not discussed here.

2.3. Image processing

Optimized VBM and subsequent analyses were carried out using SPM2 (http://www.fil.ion.ucl.ac.uk/spm/) following the methods of Good et al. (2001) and Gaser (http://dbm.neuro.uni-jena.de/vbm.html). A customized T1 template and prior probability maps of gray matter, white matter and CSF were created by combining across all study participants following the methods of Good et al. (2001). Briefly, every participant's structural MRI was normalized to the Montreal Neurological Institute (MNI) template. Normalized images were then smoothed with an 8-mm full-width half-maximum (FWHM) isotropic Gaussian kernel and averaged together to create the template. Next, participants' high resolution 3D volumes were segmented into gray and white matter images in native space and non-brain tissue was removed using an automated brain extraction procedure. The segmented images were normalized into the same stereotactic space by registering them to the study-specific custom template. The normalization parameters were reapplied to the original structural image in order to facilitate an optimal segmentation. The optimally normalized images in stereotactic space were then segmented again into gray matter, white matter, and cerebrospinal fluid using information about voxel intensity in addition to employing the prior probability maps derived from the custom templates. Segmentation also incorporated an image intensity nonuniformity correction (Ashburner and Friston, 2000) to deal with image intensity variations. The gray and white matter images were modulated (multiplied by the Jacobian determinants derived from the spatial normalization step) to compensate for volume changes in nonlinear spatial normalization. This step allows for inferences on regional volume differences between groups rather than differences in tissue density (Ashburner and Friston, 2000). All images were inspected for non-brain tissue remaining after segmentation and any non-brain tissue was removed manually. Finally, the modulated gray and white matter images were smoothed with an isotropic Gaussian kernel of 12 mm FWHM.

To correct for differences in brain size, intracranial volumes (ICV) were measured following the method described by Raz et al. (2003). Briefly, SPGR images were oriented parallel to the anterior–posterior commissural line using Analysis of Functional NeuroImages software (AFNI; http://afni.nimh.nih.gov/). Then, the ICV was manually traced on every eighth coronal section along the outer table of the cranium beginning with the orbits and continuing to the last visible posterior section of the brain.

2.4. Statistical analyses

Differences in demographics and neuropsychological scores between AI drug users and the non-AI group were assessed using Student's *t*-tests with alpha $p < 0.05$. Regional volume differences in gray and white matter between AI drug users and the non-AI group were examined using SPM2 analyses based on the general linear model. First, we tested for mean differences in gray and white matter volumes between the two groups. Second, age-related decreases in volumes were examined for each group separately. Third, the interaction between AI drug use and age-related volume decrease was tested. We hypothesized that greater age-related decreases in volume would be observed in the

Please cite this article in press as: Walther, K., et al., Anti-inflammatory drugs reduce age-related decreases in brain volume in cognitively normal older adults. Neurobiol. Aging (2009), doi:10.1016/j.neurobiolaging.2009.03.006

non-AI group compared to AI drug users. All resulting statistical maps were first thresholded at $p < 0.001$, uncorrected, and then regions with clusters of 18 or more contiguous voxels were considered significant, as demonstrated by Monte Carlo simulations carried out to estimate a whole-brain multiple comparison correction of $p < 0.05$ (Slotnick, 2008: http://www2.bc.edu/~slotnics/scripts.htm).

3. Results

The AI drug users and the non-AI group are characterized in Table 2. The two groups were well matched on demographic and cognitive variables, with no significant differences between the groups on age, education, MMSE, vocabulary, or memory or executive function scores (see Table 2).

3.1. Regional mean volume differences between AI drug users and the non-AI group

Regions of mean gray and white matter volume differences between groups were identified using whole-brain analysis of covariance (ANCOVA), with age and ICV as covariates. The results indicated several small regions of differences in gray matter between the groups. Smaller volumes in the non-AI group compared to AI drug users were detected in left superior parietal gray matter (x, y, z MNI coordinates [in mm], −10, −73, 58; $T = 4.33$; cluster size = 547 voxels) and left postcentral gyrus (x, y, z, −19, −58, 69; $T = 3.47$, cluster size = 51 voxels). In contrast, AI drug users showed smaller gray matter volumes than the non-AI group only in the left posterior lobe of the cerebellum (x, y, z, −7, −65, −51; $T = 3.22$, cluster size = 32 voxels). No regions of significant group differences were found in white matter.

3.2. Differential age-related decreases in brain volumes for the non-AI group and AI drug users

Group differences in the correlations between age and gray and white matter volumes were assessed in two stages. First, we correlated age with gray and white matter volumes, respectively, for each group including ICV as a covariate. The resulting maps show the distribution of correlation between age and gray and white matter volume in each group separately (see Fig. 1). Several brain regions showed similar negative correlations between gray matter volume and age in both groups, including the prefrontal gray matter, insula, and the thalamus. Other regions appear to be considerably smaller in extent for the AI drug group compared to the non-AI group, particularly in the frontal lobes and areas of the temporal, parietal, occipital cortex, and cerebellum. A similar pattern was observed in white matter, with similar regions of age-related decreases in the two groups surrounding the lateral ventricles. However, once again, the spatial extent of the affected regions was smaller for the AI drug users than the non-AI group.

Second, we tested for a group-by-age interaction, which would indicate those brain regions where age-related volume decreases were significantly greater in one group compared to the other. A voxel-wise multivariate regression analysis was performed in SPM2 that included group (AI drug users, non-AI group) and age as regressors and ICV as a covariate. The regions identified as significant in this analysis represent a difference in correlation of 0.60 or greater. The analysis revealed several regions of significantly greater age-related volume decreases for the non-AI group compared to the AI drug users in both gray and white matter (see Table 3). No regions showed stronger age-related decreases for the AI drug users. Areas of significant interaction within gray matter included left and right superior frontal lobe, bilateral medial precentral frontal lobe, bilateral parahippocampal gyrus, left middle and inferior temporal gyrus, fusiform gyrus, and left lateral occipital lobe. In white matter, the non-AI group showed greater age-related decreases compared to the AI drug users in the left temporal and right parietal white matter, and the left brainstem (see Fig. 2).

4. Discussion

The current study examined whether anti-inflammatory drug use could provide a protective effect against age-related changes in gray and white matter volumes, as measured by MRI. Although the VBM analyses showed greater gray matter volumes in AI drug users compared to the non-AI group in the left parietal cortex and lower gray matter volume in the left cerebellum, these mean group differences were small. In contrast, an interaction between group and age was observed that suggested a moderating effect of AI drug use on age-related decreases in brain volume. For the non-AI group, as expected, regions of both gray and white matter volumes decreased with age, while volumes in the AI drug group remained relatively stable across the age range. Specifically, the non-AI group showed significantly greater age-related volume decreases in frontal, temporal and occipital gray matter, and temporal, parietal, and cerebral peduncular white matter. No brain region showed the opposite pattern, with greater age-related volume decreases in the AI drug users compared to the non-AI group. Although the study was cross-sectional and thus cannot assess true age-related decline in brain volumes, the results are consistent with the notion that age-related decreases in brain volume that occur in cognitively normal older adults may be attenuated in individuals who have taken AI drugs for at least 2 years in the past.

One question that arises from the current results is whether the age-related volume differences observed in the non-AI group reflect a pattern more often associated with normal aging or with older adults in the early stages of Alzheimer's disease. Studies utilizing both more traditional morphometric methods as well as VBM have indicated that the frontal cortex, especially the prefrontal cortical region, is most consistently and strongly affected by normal aging

Please cite this article in press as: Walther, K., et al., Anti-inflammatory drugs reduce age-related decreases in brain volume in cognitively normal older adults. Neurobiol. Aging (2009), doi:10.1016/j.neurobiolaging.2009.03.006

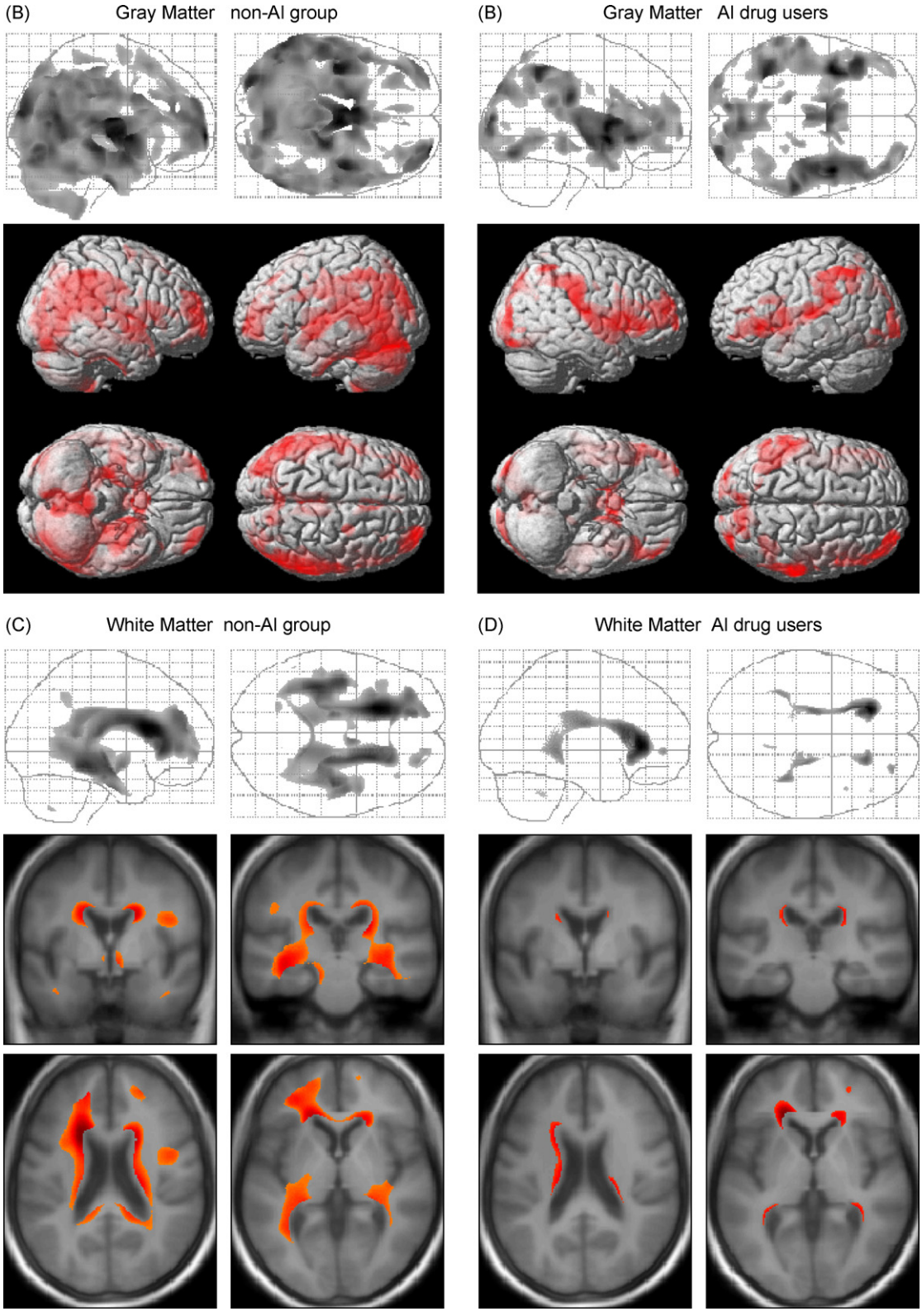

Fig. 1. Areas of significant negative correlations ($p < 0.001$) between brain volume and age for gray matter in the non-AI control group (A) and AI drug users (B) and white matter in the non-AI control group (C) and AI drug users (D). Regions of gray matter are overlaid onto glass brains and 3D surface rendered. Regions of white matter are depicted on glass brains and average T1 images at 0, −23, 2 *x*, *y*, *z* coordinates (left images) and at 0, 0, 21 *x*, *y*, *z* (right images). The pattern indicates greater areas of age-related volume differences in the non-AI group than AI drug users.

Please cite this article in press as: Walther, K., et al., Anti-inflammatory drugs reduce age-related decreases in brain volume in cognitively normal older adults. Neurobiol. Aging (2009), doi:10.1016/j.neurobiolaging.2009.03.006

Table 3
Areas of significant greater age-related gray and white matter volume decreases ($p < 0.001$) in the non-AI group compared to AI drug users.

Region	MNI coordinates (mm)			t_{value}	Cluster size[a]
	x	*y*	*z*		
Gray matter regions					
R/L medial precentral frontal	8	−19	63	3.92	2179
L middle/superior frontal	−16	7	64	4.06	1443
R superior frontal	17	−7	73	3.63	284
L superior frontal	−22	−11	69	3.36	38
L inferior temporal/parahippocampus	−41	−17	−35	3.94	1157
R parahippocampus	34	−19	−34	3.77	202
L inferior temporal/fusiform	−46	−43	−20	4.45	3600
L middle temporal	−54	−49	6	3.66	250
L lateral occipital	−30	−83	19	3.61	41
White matter regions					
L temporal	−49	−21	−9	3.42	208
R parietal	28	−51	44	3.60	226
L cerebral peduncle	−15	−15	−19	4.27	388

The coordinates of the location of maximal significance (from Montreal Neurological Institute template [MNI]), the *t* value, and the number of voxels of each cluster are provided. R: right hemisphere; L: left hemisphere.
[a] Voxel size = 1 mm × 1 mm × 1 mm.

whereas temporal (including medial temporal) and parietal cortices decline moderately across the normal adult lifespan (Alexander et al., 2006; Good et al., 2001; Raz and Rodrigue, 2006; Smith et al., 2006). In contrast, volumetric and metabolic changes are observed most often in medial temporal and parietal lobe regions in the early stages of AD as well as in cognitively normal older adults with genetic risk for AD, and these changes are more pronounced than the declines expected with normal aging (Lemaitre et al., 2005; Reiman et al., 2005; Wishart et al., 2006). In the present study, age-related decreases were relatively extensive in the non-AI group and included both frontal and temporal/parietal regions. Thus, the pattern of volume change cannot be clearly associated with either normal aging or AD.

More specifically, both groups showed age-related decreases in prefrontal gray matter that are associated with normal aging, while group differences in the degree of age-related volume decrease were prominent in the more superior and posterior portions of frontal gray matter. The precentral and superior frontal cortical regions have not been highlighted in the literature as regions typically affected in either normal aging or in the early stages of development of AD. Additionally, age-related volume decreases in the inferior and middle temporal gyrus as observed in the non-AI group have been found in previous studies of normal aging as well as in studies of adults with genetic risk for AD (Alexander et al., 2006; Lemaitre et al., 2005; Wishart et al., 2006). Finally, age-related volumetric differences in the fusiform gyrus, as observed in the non-AI group but not the AI drug users, are more consistently related to AD pathology than normal aging (Chetelat et al., 2005; Whitwell et al., 2007), although other regions that would also be expected to be affected by early AD, such as entorhinal cortex and hippocampus proper (Stoub et al., 2005; Whitwell et al., 2007) showed no age-related changes in either group. At this point, it is unclear whether the AI drug users and the non-AI group differed because anti-inflammatory drug use is protective against the early development of AD pathology, or because anti-inflammatory drugs have a more general protective effect against age-related changes in brain volume. It is important to note, however, that participants in the non-AI group were cognitively normal as indicated by extensive neuropsychological testing. Thus only a small proportion of these individuals would be expected to develop AD pathology and atrophy in brain regions associated with early AD. The current results are consistent with the notion that neuro-inflammation plays an important role in volume declines observed as part of the normal aging process (Godbout and Johnson, 2006), and that suppressing the inflammatory response with anti-inflammatory medications has a moderating effect on normal age-related decreases in both gray and white matter regions.

There are many questions that remain to be answered. Because this is a community-based sample we included participants that had taken a range of AI drugs for at least 2 years or more. The present study cannot determine which particular drug(s), or combination of drugs, might be mediating the neuroprotective effects observed here. Many of our participants were taking a combination of drugs, and over the course of their lifetimes, have gone on and off various medications for differing durations. Medications in the present study may have diverse effects, both positive and negative. For example, Vlad et al. (2008) demonstrated that ibuprofen had the strongest protective effect for AD followed by indomethacin whereas celecoxib and salicylate had no protective effect. In contrast, methotrexate when used as a chemotherapy agent in the treatment of cancer has been associated with the development of brain pathology in the form of increased white matter lesions identified on MRI (Fliessbach et al., 2005). Normally the dosages of methotrexate in cancer treatment are much higher than those taken for arthritis. Thus, in arthritis patients

Please cite this article in press as: Walther, K., et al., Anti-inflammatory drugs reduce age-related decreases in brain volume in cognitively normal older adults. Neurobiol. Aging (2009), doi:10.1016/j.neurobiolaging.2009.03.006

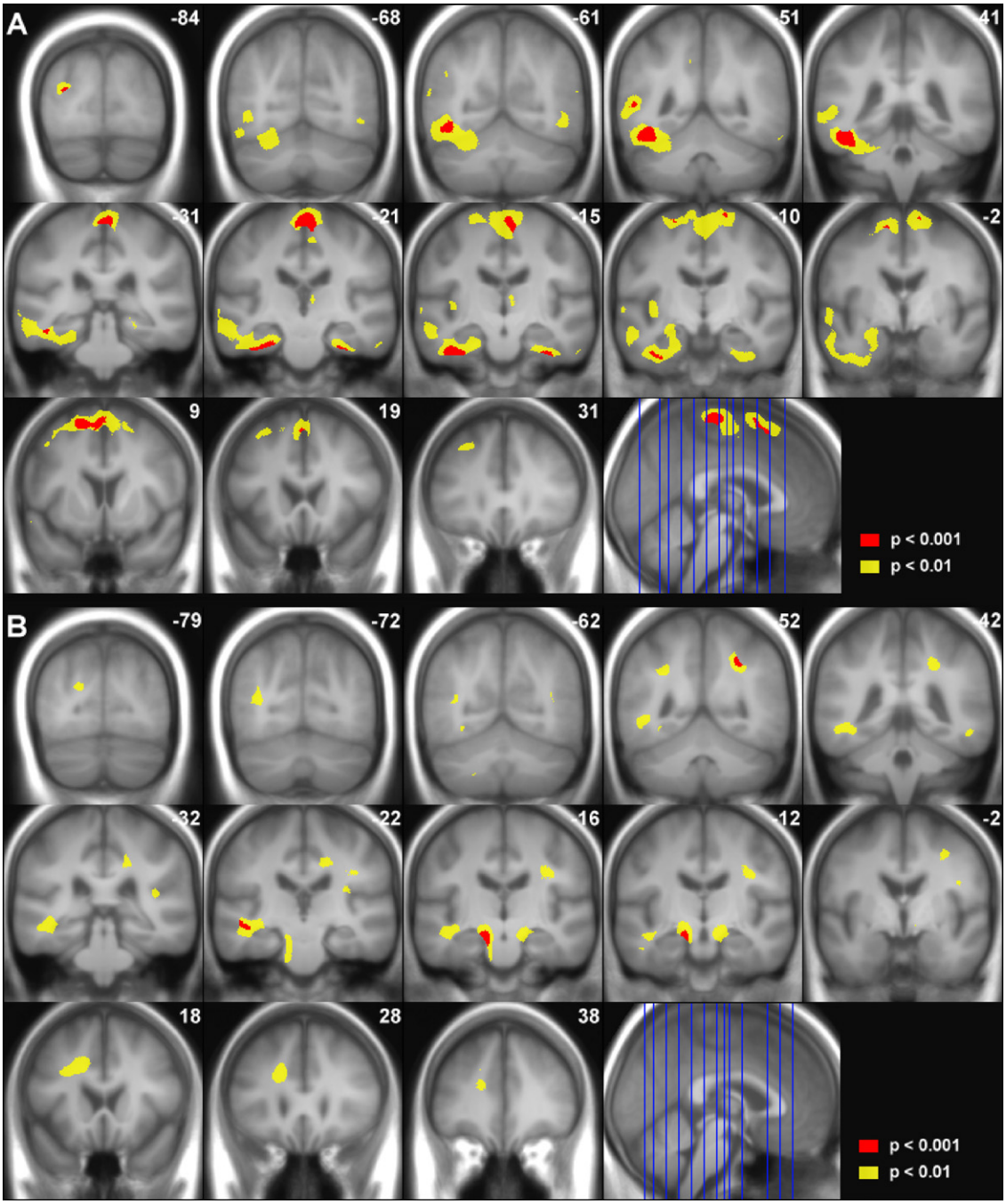

Fig. 2. Coronal sections demonstrate areas of significantly greater age-related volume differences in the non-AI group compared to AI drug users in gray matter (A) and white matter (B). Greater age-related volume differences in gray matter are detected in the superior and medial frontal gray matter, bilateral parahippocampal gyrus, left middle and inferior temporal gyri, left fusiform gyrus, and left occipital gray matter. Greater age-related volume differences are also apparent in white matter in the left temporal and right parietal lobe, and the left cerebral peduncle. Significant voxels are thresholded at $p < 0.001$ (red) and indicate a difference in correlation between the two groups of 0.60 or greater and $p < 0.01$ (yellow) indicating a difference in correlation between the two groups of 0.45 or greater. Significant regions are superimposed on the study-specific average T1 image. Locations of the coronal sections are indicated on the accompanying sagittal section.

the beneficial effect of anti-inflammation provided by AI drugs might override the neurotoxic effects of methotrexate. Little information is available on the effects of methotrexate at lower dosages. In light of the results of the present study, further studies are warranted in order to determine drug-specific effects, as well as dosages, durations, or the critical age at which AI drugs provide a benefit. All of these factors are likely to interact with one another and much larger sample sizes are needed to disentangle these specific effects.

Furthermore, AI drugs may have differential effects in individuals with and without genetic risk for AD. A recent epidemiological study conducted by Szekely et al., 2008b

Please cite this article in press as: Walther, K., et al., Anti-inflammatory drugs reduce age-related decreases in brain volume in cognitively normal older adults. Neurobiol. Aging (2009), doi:10.1016/j.neurobiolaging.2009.03.006

suggested that carriers of the APOE ε4 allele taking AI drugs had an even greater decrease in risk for developing AD compared to non-carriers. In addition, Hayden et al. (2007) reported a beneficial effect from NSAIDs on cognitive decline in APOE ε4 carriers less than 65 years of age. The APOE ε4 allele has been associated with a higher degree of brain inflammation (Jofre-Monseny et al., 2008) and thus AI drugs may be particularly effective in suppressing the inflammation and protecting against age-related cognitive and/or brain changes.

Finally, we note that the present results may be limited by the fact that only females were included in this study. Alexander et al. (2006) recently reported that men showed stronger age-related volumetric brain changes compared to women, as well as a somewhat different regional distribution of decreases. It is possible that AI drug use also might interact with gender and result in a different pattern of regions affected by AI drug use.

In summary, the use of anti-inflammatory drugs was accompanied by an attenuation of age-related volume decreases in both gray and white matter regions in older adults. The results are consistent with the hypothesis that anti-inflammatory drugs may provide protection against age-related decreases in brain volume. We believe this is the first study of its kind to demonstrate differences in age-related volumetric decreases due to AI drug use. MRI may provide an important tool for tracking and understanding the prophylactic effects of anti-inflammatory drugs in the development of AD.

Conflicts of interest

There are no actual or potential conflicts of interest.

Acknowledgements

The project was supported by the Arizona Biomedical Research Commission, the Arizona Alzheimer's Research Consortium (HB 2354, Dept. of Health Services, Arizona), and the Evelyn F. McKnight Brain Institute. We would like to thank the Arizona Arthritis Center at the University Medical Center in Tucson, Arizona for their cooperation and support. We thank Ms. Kristina Irwin for coordinating and administering neuropsychological testing.

References

ADAPT Research GroupLyketsos, C.G., Breitner, J.C., Green, R.C., Martin, B.K., Meinert, C., Piantadosi, S., Sabbagh, M., 2007. Naproxen and celecoxib do not prevent AD in early results from a randomized controlled trial. Neurology 68, 1800–1808.

Aisen, P.S., 2002. The potential of anti-inflammatory drugs for the treatment of Alzheimer's disease. Lancet Neurol. 1, 279–284.

Aisen, P.S., Schafer, K.A., Grundman, M., Pfeiffer, E., Sano, M., Davis, K.L., Farlow, M.R., Jin, S., Thomas, R.G., Thal, L.J., 2003. Effects of rofecoxib or naproxen vs placebo on Alzheimer disease progression: a randomized controlled trial. JAMA 289, 2819–2826.

Aisen, P.S., Schmeidler, J., Pasinetti, G.M., 2002. Randomized pilot study of nimesulide treatment in Alzheimer's disease. Neurology 58, 1050–1054.

Alexander, G.E., Chen, K., Merkley, T.L., Reiman, E.M., Caselli, R.J., Aschenbrenner, M., Santerre-Lemmon, L., Lewis, D.J., Pietrini, P., Teipel, S.J., Hampel, H., Rapoport, S.I., Moeller, J.R., 2006. Regional network of magnetic resonance imaging gray matter volume in healthy aging. Neuroreport 17, 951–956.

Arvanitakis, Z., Grodstein, F., Bienias, J.L., Schneider, J.A., Wilson, R.S., Kelly, J.F., Evans, D.A., Bennett, D.A., 2008. Relation of NSAIDs to incident AD, change in cognitive function, and AD pathology. Neurology 70, 2219–2225.

Ashburner, J., Friston, K.J., 2000. Voxel-based morphometry—the methods. NeuroImage 11, 805–821.

Bartolini, M., Candela, M., Brugni, M., Catena, L., Mari, F., Pomponio, G., Provinciali, L., Danieli, G., 2002. Are behaviour and motor performances of rheumatoid arthritis patients influenced by subclinical cognitive impairments? A clinical and neuroimaging study. Clin. Exp. Rheumat. 20, 491–497.

Breitner, J.C., Welsh, K.A., Helms, M.J., Gaskell, P.C., Gau, B.A., Roses, A.D., Pericak-Vance, M.A., Saunders, A.M., 1995. Delayed onset of Alzheimer's disease with nonsteroidal anti-inflammatory and histamine H2 blocking drugs. Neurobiol. Aging 16, 523–530.

Breitner, J.C., Zandi, P.P., 2001. Do nonsteroidal antiinflammatory drugs reduce the risk for Alzheimer's disease? N. Engl. J. Med. 345, 1567–1568.

Broe, G.A., Grayson, D.A., Creasey, H.M., Waite, L.M., Casey, B.J., Bennett, H.P., Brooks, W.S., Halliday, G.M., 2000. Anti-inflammatory drugs protect against Alzheimer disease at low doses. Arch. Neurol. 57, 1586–1591.

Chetelat, G., Landeau, B., Eustache, F., Mezenge, F., Viader, F., de la Sayette, V., Desgranges, B., Baron, J.C., 2005. Using voxel-based morphometry to map the structural changes associated with rapid conversion in MCI: a longitudinal MRI study. NeuroImage 27, 934–946.

Etminan, M., Gill, S., Samii, A., 2003. Effect of non-steroidal anti-inflammatory drugs on risk of Alzheimer's disease: systematic review and meta-analysis of observational studies. BMJ 327, 128.

Fliessbach, K., Helmstaedter, C., Urbach, H., Althaus, A., Pels, H., Linnebank, M., Juergens, A., Glasmacher, A., Schmidt-Wolf, I.G., Klockgether, T., Schlegel, U., 2005. Neuropsychological outcome after chemotherapy for primary CNS lymphoma: a prospective study. Neurology 64, 1184–1188.

Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J. Psychiatr. Res. 12, 189–198.

Glisky, E.L., Kong, L.L., 2008. Do young and older adults rely on different processes in source memory tasks? A neuropsychological study. J. Exp. Psychol. Learn. Mem. Cogn. 34, 809–822.

Godbout, J.P., Johnson, R.W., 2006. Age and neuroinflammation: a lifetime of psychoneuroimmune consequences. Neurol. Clin. 24, 521–538.

Good, C.D., Johnsrude, I.S., Ashburner, J., Henson, R.N., Friston, K.J., Frackowiak, R.S., 2001. A voxel-based morphometric study of ageing in 465 normal adult human brains. NeuroImage 14, 21–36.

Hayden, K.M., Zandi, P.P., Khachaturian, A.S., Szekely, C.A., Fotuhi, M., Norton, M.C., Tschanz, J.T., Pieper, C.F., Corcoran, C., Lyketsos, C.G., Breitner, J.C., Welsh-Bohmer, K.A., 2007. Does NSAID use modify cognitive trajectories in the elderly? The Cache County study. Neurology 69, 275–282.

Henderson, A.S., Jorm, A.F., Christensen, H., Jacomb, P.A., Korten, A.E., 1997. Aspirin, anti-inflammatory drugs and risk of dementia. Int. J. Geriatr. Psychiatry 12, 926–930.

in't Veld, B.A., Launer, L.J., Hoes, A.W., Ott, A., Hofman, A., Breteler, M.M., Stricker, B.H., 1998. NSAIDs and incident Alzheimer's disease. The Rotterdam study. Neurobiol. Aging 19, 607–611.

Please cite this article in press as: Walther, K., et al., Anti-inflammatory drugs reduce age-related decreases in brain volume in cognitively normal older adults. Neurobiol. Aging (2009), doi:10.1016/j.neurobiolaging.2009.03.006

in't Veld, B.A., Ruitenberg, A., Hofman, A., Launer, L.J., van Duijn, C.M., Stijnen, T., Breteler, M.M., Stricker, B.H., 2001. Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease. N. Engl. J. Med. 345, 1515–1521.

Jofre-Monseny, L., Minihane, A.M., Rimbach, G., 2008. Impact of apoE genotype on oxidative stress, inflammation and disease risk. Mol. Nutr. Food Res. 52, 131–145.

Lemaitre, H., Crivello, F., Dufouil, C., Grassiot, B., Tzourio, C., Alperovitch, A., Mazoyer, B., 2005. No epsilon4 gene dose effect on hippocampal atrophy in a large MRI database of healthy elderly subjects. NeuroImage 24, 1205–1213.

McGeer, P.L., McGeer, E.G., Rogers, J., Sibley, J., 1990. Anti-inflammatory drugs and Alzheimer disease. Lancet 335, 1037.

McGeer, P.L., Schulzer, M., McGeer, E.G., 1996. Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. Neurology 47, 425–432.

Mechelli, A., Friston, K.J., Frackowiak, R.S., Price, C.J., 2005. Structural covariance in the human cortex. J. Neurosci. 25, 8303–8310.

Raz, N., Rodrigue, K.M., 2006. Differential aging of the brain: patterns, cognitive correlates and modifiers. Neurosci. Biobehav. Rev. 30, 730–748.

Raz, N., Rodrigue, K.M., Acker, J.D., 2003. Hypertension and the brain: vulnerability of the prefrontal regions and executive functions. Behav. Neurosci. 117, 1169–1180.

Reiman, E.M., Chen, K., Alexander, G.E., Caselli, R.J., Bandy, D., Osborne, D., Saunders, A.M., Hardy, J., 2005. Correlations between apolipoprotein E epsilon4 gene dose and brain-imaging measurements of regional hypometabolism. Proc. Natl. Acad. Sci. U.S.A. 102, 8299–8302.

Reines, S.A., Block, G.A., Morris, J.C., Liu, G., Nessly, M.L., Lines, C.R., Norman, B.A., Baranak, C.C., Rofecoxib Protocol 091 Study Group, 2004. Rofecoxib: no effect on Alzheimer's disease in a 1-year, randomized, blinded, controlled study. Neurology 62, 66–71.

Scharf, S., Mander, A., Ugoni, A., Vajda, F., Christophidis, N., 1999. A double-blind, placebo-controlled trial of diclofenac/misoprostol in Alzheimer's disease. Neurology 53, 197–201.

Slotnick, S.D., 2008. Cluster_threshold. Retrieved December 10, 2008 from http://www2.bc.edu/~slotnics/scripts.htm.

Smith, C.D., Chebrolu, H., Wekstein, D.R., Schmitt, F.A., Markesbery, W.R., 2006. Age and gender effects on human brain anatomy: a voxel-based morphometric study in healthy elderly. Neurobiol. Aging 28, 1075–1087.

Stoub, T.R., Bulgakova, M., Leurgans, S., Bennett, D.A., Fleischman, D., Turner, D.A., Toledo-Morrell, L., 2005. MRI predictors of risk of incident Alzheimer disease: a longitudinal study. Neurology 64, 1520–1524.

Szekely, C.A., Thorne, J.E., Zandi, P.P., Ek, M., Messias, E., Breitner, J.C., Goodman, S.N., 2004. Nonsteroidal anti-inflammatory drugs for the prevention of Alzheimer's disease: a systematic review. Neuroepidemiology 23, 159–169.

Szekely, C.A., Green, R.C., Breitner, J.C., Ostbye, T., Beiser, A.S., Corrada, M.M., Dodge, H.H., Ganguli, M., Kawas, C.H., Kuller, L.H., Psaty, B.M., Resnick, S.M., Wolf, P.A., Zonderman, A.B., Welsh-Bohmer, K.A., Zandi, P.P., 2008a. No advantage of A beta 42-lowering NSAIDs for prevention of Alzheimer dementia in six pooled cohort studies. Neurology 70, 2291–2298.

Szekely, C.A., Breitner, J.C., Fitzpatrick, A.L., Rea, T.D., Psaty, B.M., Kuller, L.H., Zandi, P.P., 2008b. NSAID use and dementia risk in the Cardiovascular Health Study: role of APOE and NSAID type. Neurology 70, 17–24.

Tuppo, E.E., Arias, H.R., 2005. The role of inflammation in Alzheimer's disease. Int. J. Biochem. Cell. Biol. 37, 289–305.

Vlad, S.C., Miller, D.R., Kowall, N.W., Felson, D.T., 2008. Protective effects of NSAIDs on the development of Alzheimer disease. Neurology 70, 1672–1677.

Wechsler, D., 1999. Wechsler Abbreviated Scale of Intelligence (WASI). San Antonio, PsychCorp.

Whitwell, J.L., Przybelski, S.A., Weigand, S.D., Knopman, D.S., Boeve, B.F., Petersen, R.C., Jack Jr, C.R., 2007. 3D maps from multiple MRI illustrate changing atrophy patterns as subjects progress from mild cognitive impairment to Alzheimer's disease. Brain 130, 1777–1786.

Wishart, H.A., Saykin, A.J., McAllister, T.W., Rabin, L.A., McDonald, B.C., Flashman, L.A., Roth, R.M., Mamourian, A.C., Tsongalis, G.J., Rhodes, C.H., 2006. Regional brain atrophy in cognitively intact adults with a single APOE epsilon4 allele. Neurology 67, 1221–1224.

Please cite this article in press as: Walther, K., et al., Anti-inflammatory drugs reduce age-related decreases in brain volume in cognitively normal older adults. Neurobiol. Aging (2009), doi:10.1016/j.neurobiolaging.2009.03.006