ANNALS of Clinical and Translational Neurology
Open Access
AMERICAN NEUROLOGICAL ASSOCIATION

RESEARCH ARTICLE

Associations of renin–angiotensin system inhibitor use with brain insulin signaling and neuropathology

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Received: 4 June 2024; Accepted: 6 June 2024

Annals of Clinical and Translational Neurology **2024; 11(8): 2112–2122**

doi: 10.1002/acn3.52132

Abstract

Objective: To examine the associations of renin–angiotensin system (RAS) inhibitor use with postmortem brain insulin signaling and neuropathology. **Methods**: Among Religious Orders Study participants, 150 deceased and autopsied older individuals (75 with diabetes matched to 75 without by age at death, sex, and education) had measurements of insulin receptor substrate-1 (IRS-1) and RAC-alpha serine/threonine protein kinase (AKT1) collected in the prefrontal cortex using ELISA and immunohistochemistry. Alzheimer's disease (AD), brain infarcts, and cerebral vessel pathology data were assessed by systematic neuropathologic evaluations. RAS inhibitor use was determined based on visual inspection of medication containers during study visits. The associations of RAS inhibitor use with brain insulin signaling measures and neuropathology were examined using adjusted regression analyses. **Results**: Of the 90 RAS inhibitor users (54 with diabetes), 65 had used only angiotensin-converting enzyme inhibitors, 11 only angiotensin II receptor blockers, and 14 used both. RAS inhibitor use was associated with lower pT^{308}AKT1/total AKT1, but not with pS^{307}IRS-1/total IRS-1 or the density of cells stained positive for pS^{616} IRS-1. RAS inhibitor use was not associated with the level of global AD pathology or amyloid beta burden, but it was associated with a lower tau-neurofibrillary tangle density. Additionally, we found a significant interaction between diabetes and RAS inhibitors on tangle density. Furthermore, AKT1 phosphorylation partially mediated the association of RAS inhibitor use with tau tangle density. Lastly, RAS inhibitor use was associated with more atherosclerosis, but not with other cerebral blood vessel pathologies or cerebral infarcts. **Interpretation**: Late-life RAS inhibitor use may be associated with lower brain AKT1 phosphorylation and fewer neurofibrillary tangles.

Introduction

Renin–angiotensin system (RAS) inhibitors, namely angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs), are commonly prescribed first-line antihypertensives. They are particularly recommended to be used in patients with comorbid hypertension and type-2 diabetes mellitus (T2DM),[1] a population at increased risk for dementia.[2] Accumulating evidence suggests that RAS inhibitor use may be associated with a lower risk of cognitive decline and dementia beyond the benefit of blood pressure reduction.[3] Notably, one recent study found that older individuals with mild cognitive impairment (MCI) who had used RAS inhibitors exhibited slower disease progression over time and less postmortem Alzheimer's disease (AD) pathology than nonusers.[4] However, the mechanisms underlying the protective effect of RAS inhibitor use on cognition are still unclear.

As the drug target of RAS inhibitors, angiotensin II is known to inhibit insulin-mediated phosphoinositide 3-

kinase/protein kinase B (PI3K/AKT) pathway activation, thereby contributing to insulin resistance,[5] a major pathophysiological feature of T2DM. In addition, multiple clinical trials have shown that, compared to nonusers, RAS inhibitor users in patients with hypertension tend to have increased insulin sensitivity[6,7] and a lower incidence of new-onset diabetes.[8] Given that altered brain insulin signaling (specifically, increased phosphorylation of RAC-alpha serine/threonine protein kinase [AKT1]) has been found to be associated with lower late-life cognition and more severe pathology of AD and cerebrovascular disease in previous studies by our group,[9,10] it is tempting to speculate that improving insulin signaling in the brain might be one pathway linking RAS inhibitor use to a decreased risk for dementia. Nevertheless, whether RAS inhibitor use is associated with brain insulin signaling and accumulation of dementia-related neuropathology remains obscure.

Here, we examine the associations of RAS inhibitor use, mainly ACE inhibitors, with brain insulin signaling molecules (including insulin receptor substrate 1 [IRS-1] and AKT1) and the severity of common neuropathology of dementia. To this end, we included data collected from antemortem clinical evaluations and postmortem brain specimens of 150 older individuals (75 with diabetes matched to 75 without) who were part of a community-based clinical-pathological cohort (the Religious Orders Study), and who had brain insulin signaling data available. Levels of brain insulin signaling molecules in the prefrontal cortex were assessed postmortem using enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry approaches. The severity of neuropathology, including AD, brain infarcts, and cerebral vessels, was also assessed. RAS inhibitor use was determined based on annual clinical evaluations. By using adjusted mixed-effects regression analyses, we tested the hypothesis that RAS inhibitor use during the study was associated with a lower level of brain insulin resistance and less severe neuropathology.

Subjects and Methods

Participants

We used data from participants of the Religious Orders Study (ROS), an ongoing, prospective clinical–pathological cohort study of aging.[11] Approved by the Rush University Institutional Review Board, ROS began enrolling Catholic clergy (nuns, priests, and brothers) in 1994 from convents and monasteries across the United States. All study participants signed an informed consent form to undergo annual medical and neuropsychological evaluation, and an anatomical gift act to donate the brain after death. They signed a repository consent allowing their resources to be shared.

Clinical evaluations

The participants underwent annual clinical evaluations including medical history, physical examination, and a detailed assessment of cognitive function using neuropsychological tests, as described previously.[9] All participants were required to bring prescription and over-the-counter medication containers, which were visually inspected and coded using the Medi-Span system.[12] RAS inhibitor users were defined as those who were found to be using ACE inhibitors and ARBs at baseline visit or in at least one follow-up cycle. The presence of diabetes was determined based on either medical history or visually inspected containers of antidiabetic medications, as previously published.[13]

Brain autopsy procedure and neuropathological data

Brain autopsies were performed at predetermined sites across the United States following a standardized procedure, as previously described.[14] Briefly, the brains of the deceased participants were removed and weighed, and the cerebral hemispheres were cut into 1 cm coronal slabs. Slabs not designated for freezing were fixed in 4% paraformaldehyde for 3–21 days before the macroscopic examination for infarcts and the dissection of diagnostic blocks. The blocks were paraffin-embedded, cut into 6 μm sections, and mounted on microscope slides.

Neuropathological diagnoses were made by a board-certified neuropathologist blinded to clinical data. For the diagnosis of AD pathology, a modified Bielschowsky silver stain was used to visualize neuritic plaques, diffuse plaques, and neurofibrillary tangles, and the counts of these three neuropathological markers within 1 mm^2 area from sections of midfrontal, superior temporal, inferior parietal, and entorhinal cortices were summarized in one continuous, standardized, global AD pathology measure, as previously described.[15] In addition, quantitative measures of amyloid burden and neurofibrillary tangle density were obtained using immunohistochemistry with antibodies to amyloid-β and PHF-tau.[16] Given the non-normal distribution of these measures, square root transformed data were used for analyses. For the diagnosis of cerebrovascular disease, gross infarcts were identified on macroscopic examination and classified by number, location (cortical, subcortical), and volume (in mm^2). Each gross infarct was then dissected and confirmed on microscopic examination, and classified by age (acute, subacute, chronic). Micro-infarcts were identified under microscope in blocks of nine brain regions that were stained with H&E, and their location and age were also recorded.[17] Only chronic infarcts were considered in this study.

Cerebral vessel pathology was also examined systematically, as previously published.[18] Briefly, atherosclerosis severity was graded using a semiquantitative scale from 0 (no atherosclerosis) to 6 (severe atherosclerosis) following visual inspection of vessels in the circle of Willis. Similarly, arteriolosclerosis severity was graded using a scale from 0 (no arteriolosclerosis) to 7 (complete small vessel occlusion) during histologic examination of the anterior basal ganglia. Last, amyloid angiopathy severity was graded in several neocortical brain regions, based on the degree of immunohistochemical labeling with anti-amyloid-β.[19]

Case selection and brain insulin signaling measures

Among all deceased ROS participants who came to the autopsy, we selected 150 cases with completed data collection and brain tissue samples available for this study, using rigorously applied inclusion and exclusion criteria, as recently described elsewhere.[9] Briefly, in order to obtain a spectrum across insulin resistance and allow for examination of differential effects by diabetes status, we previously selected 75 subjects with and 75 without diabetes, matched by sex, age at death, and years of education.

Brain insulin signaling measures were quantified in the dorsolateral prefrontal cortex (DLPFC) within the middle frontal gyrus cortex (MFC), as described elsewhere in detail.[9] Total IRS1 and phosphorylated IRS1 at Ser307 (pS^{307}IRS1), and total AKT1 and phosphorylated AKT1 at Thr308 (pT^{308}IRS1), were quantified using PathScan enzyme-linked immunosorbent assay (ELISA) kits (Cell Signaling Technology, Danvers, MA) based on the traditional solid-phase sandwich ELISA method. The catalog numbers for the ELISA kits were as follows: total IRS1, #7328; pS^{307}IRS1, #7287; AKT1, #7170; and pT^{308}AKT1, #7252. The pS^{307}IRS1/total IRS1 and pT^{308}AKT1/total AKT1 ratios were calculated to estimate IRS1 phosphorylation and AKT1 phosphorylation, respectively. In addition, phosphorylated IRS1 at Ser616 (pS^{616}IRS1) were assessed in 10 μm-thick MFC tissue sections using immunohistochemistry, as previously described.[9] Primary antibody against pS^{616}IRS (44550G, Invitrogen, Carlsbad, CA; rabbit 1:500) was used for incubation of pretreated tissues overnight at 4°C. Next, sections were incubated in secondary antibody followed by avidin–biotin–peroxidase complex for 1 h and finally reacted with a 0.05% diaminobenzidine (DAB)–0.03% hydrogen peroxide solution for 10 min. The immunoreaction signal was enhanced by adding NiSO4 (0.25% final dilution) to the DAB solution. We used high-throughput computer-assisted image analysis count determinations to quantify pS^{616}IRS1. A semiquantitative variable of the number of pS^{616}IRS1 cell profiles/mm^2 was used for further analyses.

Statistical approach

Data were first characterized (descriptive analyses), and plots were generated, to examine for outliers and skewness of data, among other features. First, we fit separate linear regression models with brain insulin signaling measures (pS^{307}IRS1/total IRS1, pT^{308}AKT1/total AKT1, and pS^{616}IRS1 cells per mm^2) as outcomes with terms for RAS inhibitor use, age at death, sex, and diabetes status. Next, we fit models with neuropathological outcomes, starting with separate linear regression models for AD pathology (scores for global AD pathology, and specifically for amyloid burden and tau tangle density). Then, logistic regressions were used to examine the relation of RAS inhibitor use with cerebrovascular pathology, including the presence (versus absence) of infarcts (any infarcts, gross infarcts, micro-infarcts, cortical infarcts, and subcortical infarcts) and the severity (two-levels: none or mild, versus moderate or severe) of cerebral blood vessel pathology (atherosclerosis, arteriolosclerosis, amyloid angiopathy). All models adjusted for age at death, sex, and diabetes, and age at death was centered on the mean for interpretation purposes. Analyses were conducted using SAS/STAT software, version 9.4 of the SAS system for Linux (SAS Institute, Cary, NC). A two-tailed hypothesis was assumed.

Results

Sample characteristics

The demographic, clinical, and neuropathologic characteristics of the 150 study participants have been reported in a previous study.[9] To summarize, the mean age at death was slightly over 86 years, and nearly half of study participants were female. On average, participants had just over 18 years of education. There was no difference among participants with and without diabetes in demographic characteristics (age at death, sex, and education), in the level of cognitive function measured within 1 year before death and in the severity of postmortem AD pathology (all $p > 0.05$). The mean duration of follow-up was 9.4 years.

The demographic and clinical characteristics of study participants by RAS inhibitor use are summarized in Table 1. Of 150 participants included in this study, 90 were users of RAS inhibitors, and 60 were nonusers. We did not find a difference between RAS inhibitor users and nonusers in age at death, sex, or years of education. Compared with nonusers, a higher percentage of RAS inhibitor users had a history of hypertension, coronary heart disease, and diabetes. Mean blood pressure and body mass index (BMI) levels were similar between these two groups. Of the 90 RAS inhibitor users, data across the study

Table 1. Demographic and clinical characteristics of study participants by RAS inhibitor use.

Participant characteristics*	Total (n = 150)	RAS inhibitor users (n = 90)	RAS inhibitor nonusers (n = 60)
Demographics			
Age at death, years	86.6 (6.1)	86.8 (5.9)	86.3 (6.4)
Female, *n* (%)	72.0 (48.0%)	44.0 (48.9%)	28.0 (46.7%)
Education, years	18.2 (3.3)	17.9 (3.0)	18.5 (3.6)
Medical conditions			
Diabetes, *n* (%)*	75.0 (50.0%)	54.0 (60.0%)	21.0 (35.0%)
Hypertension, *n* (%)*	105.0 (70.0%)	73.0 (81.1%)	32.0 (53.3%)
Coronary heart disease, *n* (%)*	43.0 (28.7%)	32.0 (35.6%)	11.0 (18.3%)
Stroke, *n* (%)	38.0 (25.3%)	22.0 (24.4%)	16.0 (26.7%)
Physical examination			
Systolic blood pressure, mmHg	123.3 (15.9)	124.5 (16.6)	121.68 (15.4)
Diastolic blood pressure, mmHg	69.3 (8.3)	67.1 (8.5)	72.3 (7.4)
Body mass index, kg/m^2	26.4 (5.7)	26.6 (5.6)	26.2 (5.9)

Mean (SD) unless specified. An asterisk (*) indicates statistical significance ($p < 0.05$) between RAS inhibitor users and nonusers in a two-sample *t*-test or a Chi-square test.

showed that 65 had used only ACE inhibitors but not ARBs, 11 had used only ARBs but not ACE inhibitors, and 14 had used both ACE inhibitors and ARBs. In addition, 79 had used RAS inhibitors in at least one follow-up cycle in their last 5 years prior to death. Furthermore, the vast majority of RAS inhibitor users (87/90) also had used other classes of antihypertensives, including diuretics, beta-blockers, and calcium-channel blockers. Of the 60 RAS inhibitor nonusers, 17 had not used any antihypertensives. In addition, no difference was found in AD global pathology score, amyloid burden, or tau tangle density between RAS inhibitor users and nonusers (data not shown).

Associations of RAS inhibitor use with brain insulin signaling measures

First, we examined the associations of RAS inhibitor use with postmortem brain insulin signaling measures using linear regression models adjusting for age at death, sex, and diabetes. As shown in Table 2, we found that RAS inhibitor use at any time during the study was associated with a lower level of pT^{308}AKT1/total AKT1. This association remained significant after additionally adjusting for (i) a summary measure of vascular risk factors based on hypertension and smoking (estimate = −0.357, SE = 0.171, p = 0.039), and (ii) both mean systolic blood pressure and mean diastolic blood pressure (estimate = −0.435, SE = 0.170, p = 0.011). We did not find an association between RAS inhibitor use during the study with pS^{307}IRS1/total IRS1 or the density of pS^{616}IRS1 stained cells. In secondary analyses, RAS inhibitor use during the last 5 years of life was associated with a lower level of pS^{307}IRS1/total IRS1 as well as a lower level of pT^{308}AKT1/total AKT1, but not with the density of pS^{616}IRS1 stained cells. In addition, we found that ACE inhibitor use at any time during the study was also associated with a lower level of pT^{308}AKT1/total AKT1 (estimate = −0.350, SE = 0.164, p = 0.035), but not with the other two insulin signaling measures (data not shown). We did not find an association of ACE inhibitor use during the last 5 years of life with any of the brain insulin signaling measures (data not shown). We did not find an interaction effect of diabetes with RAS inhibitor use (Table S1) or ACE inhibitor use (data not shown) on brain insulin signaling measures.

Table 2. Associations of RAS inhibitor use with brain insulin signaling measures.

	Estimate (SE, *p*-value)		
RAS inhibitor use	pS^{307}IRS1/total IRS1	pT^{308}AKT1/total AKT1	pS^{616}IRS1 cells/mm^2
During the study	−0.244 (0.164, 0.139)	**−0.397 (0.168, 0.019)**	0.073 (0.180, 0.685)
During last 5 years of life	**−0.320 (0.158, 0.045)**	**−0.340 (0.163, 0.039)**	0.082 (0.175, 0.641)

Each linear regression model with individual brain insulin signaling marker as the outcome has been adjusted for age at death, sex, and diabetes. Bold values denote statistical significance ($p < 0.05$).

Associations of RAS inhibitor use with the severity of AD pathology

Next, we examined associations of RAS inhibitor use with the severity of AD pathology (Table 3). We did not find associations of RAS inhibitor use during the study with global AD score or amyloid beta burden. However, we found that RAS inhibitor use during the study was associated with lower tau tangle density.

The association between RAS inhibitor use during the study and tau tangle density remained significant after additionally adjusting for a summary measure of hypertension and smoking combined (estimate = −0.494, SE = 0.186, $p = 0.009$), and in a separate model for the mean systolic blood pressure and mean diastolic blood pressure (estimate = −0.427, SE = 0.186, $p = 0.023$). In secondary analyses, we did not find an association of RAS inhibitor use during the last 5 years of life with any of the AD pathology measures. Likewise, we did not find an association of ACE inhibitor use during the study or during the last 5 years of life with any of the AD pathology measures (data not shown).

Moreover, we found significant interaction effects of diabetes with RAS inhibitor use during the study on the global AD pathology score, and specifically tau tangle density (Table 4). In additional models with the global AD pathology outcome, we continued to observe an interaction of RAS inhibitor use during the study with diabetes after adjusting for combined vascular risk factors of hypertension and smoking (estimate = −0.590, SE = 0.195, $p = 0.003$) and separately, for both systolic and diastolic blood pressures (estimate = −0.593, SE = 0.195, $p = 0.003$). Likewise, there was an interaction of RAS inhibitors with diabetes on tau tangle density after adjusting for vascular risk factors (estimate = −1.177, SE = 0.355, $p = 0.001$) and for blood pressure (estimate = −1.191, SE = 0.355, $p = 0.001$). In secondary analyses, RAS inhibitor use during the last 5 years of life showed similar interactions with diabetes on both global AD and tau tangle density. In the same way, ACE inhibitor use during the study interacted with diabetes on global AD score (estimate = −0.473, SE = 0.192, $p = 0.015$) and tau tangle density (estimate = −0.960, SE = 0.356, $p = 0.008$). ACE inhibitor use during the last 5 years of life also had a differential effect in the presence of diabetes on global AD score and tau tangle density (estimate of the interaction term respectively −0.457, SE = 0.192, $p = 0.019$; and −0.919, SE = 0.360, $p = 0.012$). To better understand these interactions, we further conducted stratified analyses by diabetes status. We found that RAS inhibitor use during the study was associated with a lower global AD score only in participants with diabetes (estimate = −0.471, SE = 0.139, $p = 0.001$), but not in those without diabetes (estimate = 0.100, SE = 0.133, $p = 0.456$). Similarly, RAS inhibitor use during the study was associated with fewer tau tangles in participants with diabetes (estimate = −1.039, SE = 0.247, $p < 0.001$), but not in those without (estimate = 0.095, SE = 0.260, $p = 0.716$).

To explore the effect of AKT1 phosphorylation on the association of RAS inhibitor use during the study with tau tangle density, we added pT^{308}AKT1/total AKT1 as a covariate to the regression model. We found that lower AKT1 phosphorylation was associated with a lower tau tangle density (estimate = 0.222, SE = 0.090, $p = 0.014$), as we reported previously.[9] However, the association between

Table 3. Associations of RAS inhibitor use with AD pathology.

	Estimate (SE, *p*-value)		
RAS inhibitor use	Global AD score	Amyloid burden	Tau tangle density
During the study	−0.165 (0.100, 0.099)	−0.048 (0.170, 0.780)	**−0.425 (0.184, 0.022)**
During last 5 years of life	−0.093 (0.097, 0.338)	−0.148 (0.164, 0.369)	−0.245 (0.180, 0.177)

Each linear regression model with individual AD pathology measure as the outcome has been adjusted for age at death, sex, and diabetes. Bold values denote statistical significance ($p < 0.05$).

Table 4. Interaction effects of RAS inhibitor use with diabetes on AD pathology.

	Estimate (SE, *p*-value)		
RAS inhibitor use	Global AD score	Amyloid burden	Tau tangle density
During the study	**−0.593 (0.195, 0.003)**	−0.620 (0.339, 0.069)	**−1.174 (0.358, 0.001)**
During last 5 years of life	**−0.450 (0.190, 0.019)**	−0.538 (0.327, 0.102)	**−0.884 (0.355, 0.014)**

Each linear regression model with individual AD pathology measure as the outcome has been adjusted for age at death, sex, and diabetes. Bold values denote statistical significance ($p < 0.05$).

RAS inhibitor use and tau tangle density was no longer significant (estimate = −0.337, SE = 0.184, $p = 0.069$), with a reduction of 20.7% in effect size. This suggests that AKT1 phosphorylation partially mediates the association of RAS inhibitor use and tau tangle density.

Associations of RAS inhibitor use with the presence of brain infarcts

We also examined the associations of RAS inhibitor use with the presence of brain infarcts. We did not find any associations of RAS inhibitor use during the study with the presence of any brain infarcts, gross infarcts, micro-infarcts, cortical infarcts, and subcortical infarcts (Table 5). In secondary analyses, we found that RAS inhibitor use during the last 5 years of life was associated with approximately 2.6 times increased odds of developing micro-infarcts, but not with the presence of other types of brain infarcts. We did not find an association of ACE inhibitor use during the study or the last 5 years of life with the presence of any type of brain infarcts (data not shown). We did not find any interaction effect of diabetes with RAS inhibitor use (Table S2) or ACE inhibitor use (data not shown) on the presence of brain infarcts.

Associations of RAS inhibitor use with the severity of cerebral vessel pathology

Last, we examined the associations of RAS inhibitor use with the severity of cerebral vessel pathology. RAS inhibitor use during the study and during the last 5 years of life was both associated with approximately 2.1 times increased odds of developing moderate or severe atherosclerosis (Table 6), but not with the severity of arteriolosclerosis or amyloid angiopathy. The association between RAS inhibitor use during the study with the presence of moderate to severe atherosclerosis was no longer significant after additionally adjusting for a summary variable of vascular risk factors based on hypertension and smoking (OR = 1.786, estimate = 0.580, SE = 0.376, $p = 0.122$) and for mean systolic and diastolic blood pressures (OR = 1.941, estimate = 0.663, SE = 0.371, $p = 0.074$). This suggests that the association we previously identified was likely explained by blood pressure control. In a secondary analysis, although ACE inhibitor use during the study was not associated with the severity of any cerebral vessel pathology (data not shown), ACE inhibitor use during the last 5 years of life was associated with 2.6 times increased odds of developing moderate or severe atherosclerosis (OR = 2.605, estimate = 0.958, SE = 0.355, $p = 0.007$). We did not find any interaction effect of diabetes with RAS inhibitor use (Table S3) or ACE inhibitor use (data not shown) on the severity of cerebral vessel pathology.

Discussion

In this study, we examined the associations of RAS inhibitor use with postmortem brain insulin signaling and neuropathology of aging and dementia, in 150 community-dwelling older persons with diabetes matched to persons

Table 5. Association of RAS inhibitor use with brain infarcts.

	OR, Estimate (SE, *p*-value)				
		Size of infarct		Location of infarct	
RAS inhibitor use	Any infarct	Gross infarcts	Micro-infarcts	Cortical infarcts	Subcortical infarcts
During the study	1.294, 0.258 (0.353, 0.465)	1.315, 0.274 (0.367, 0.457)	1.584, 0.460 (0.392, 0.240)	1.480, 0.392 (0.424, 0.356)	1.480, 0.392 (0.372, 0.292)
During last 5 years of life	1.812, 0.594 (0.344, 0.084)	1.564, 0.447 (0.356, 0.209)	**2.579, 0.947 (0.385, 0.014)**	2.2436, 0.808 (0.418, 0.053)	1.805, 0.591 (0.360, 0.101)

Each linear regression model with individual measure of brain infarcts as the outcome has been adjusted for age at death, sex, and diabetes. Bold values denote statistical significance ($p < 0.05$).

Table 6. Association of RAS inhibitor use with cerebral vessel pathologies.

	OR, Estimate (SE, *p*-value)		
RAS inhibitor use	Atherosclerosis	Arteriolosclerosis	Amyloid angiopathy
During the study	**2.056, 0.721 (0.363, 0.047)**	0.551, −0.595 (0.375, 0.113)	0.829, −0.188 (0.380, 0.621)
During last 5 years of life	**2.129, 0.756 (0.350, 0.031)**	0.535, −0.626 (0.368, 0.089)	0.897, −0.109 (0.370, 0.769)

Each linear regression model with individual measure of cerebral vessel pathologies as the outcome has been adjusted for age at death, sex, and diabetes. Bold values denote statistical significance ($p < 0.05$).

without diabetes. We found that RAS inhibitor use was associated with a measure of insulin signaling, and specifically with a lower level of brain AKT1 phosphorylation at position Thr^{308}. With neuropathology outcomes, there was no association of RAS inhibitor use with a global AD score or with the specific marker for amyloid burden. However, RAS inhibitors were associated with a lower density of neurofibrillary tangles, particularly in persons with diabetes. In addition, we found that AKT1 phosphorylation partially mediates the association of RAS inhibitor use and tau tangle density. Lastly, RAS inhibitors were associated with some measures of cerebrovascular neuropathology. While RAS inhibitors were not associated with brain infarcts as a whole, we found increased odds of moderate or severe atherosclerosis in RAS inhibitor users vs. nonusers. Taken together, these findings suggest that late-life RAS inhibitor use is associated with brain insulin signaling and some measures of neurodegenerative and cerebrovascular neuropathology.

Converging lines of evidence suggest that Ang II, the key effector of the RAS, plays a pivotal role in the development of insulin resistance,[20,21] and that RAS inhibitors, agents that inhibit the action of Ang II, can improve insulin sensitivity.[22,23] In animal studies, although acute stimulation with Ang II induces the tyrosine phosphorylation of IRS-1 and inhibits the insulin signaling pathway,[24] chronic infusion of Ang II into normal rats induces hypertension and insulin resistance, accompanied by enhanced insulin-induced PI3K activation and AKT phosphorylation.[25] In addition, the treatment with tempol, a superoxide dismutase mimetic, normalized insulin resistance and restored PI3K activation. This suggests that Ang II-induced insulin resistance is attributed to oxidative stress, possibly through impaired insulin signaling located downstream of PI3K-AKT1 activation.[25] However, these studies were conducted in animal models, and using endothelial cells, muscle cells, or adipocytes. To our knowledge, no previous study has investigated the effect of Ang II or Ang II blockade via RAS inhibitors on insulin signaling in the human brain. In our prior work on brain insulin signaling, using the same sample as in this study, we demonstrated that a higher level of AKT1 phosphorylation at Thr^{308} in postmortem brain was not only associated with a lower level of global cognition approximate to death,[9] but also associated with a faster late-life cognitive decline.[26] The underlying mechanisms may involve abnormal interactions between AKT1 and downstream glycogen synthesis kinase 3 β (GSK3β), which may promote the accumulation of paired-helical filament tau and some suggest of also amyloid-β plaques, signature pathologies of AD.[27] Moreover, persistent AKT1 activation can indirectly activate the mammalian target of rapamycin complex 1 (mTORC1), which inhibits the upstream IRS1 and further contributes to brain insulin resistance by forming a vicious cycle.[28] Consistent with the findings of previous studies, our results in this study further established the association of late-life RAS inhibitor use with lower AKT1 phosphorylation in postmortem brain, which has been linked to a high level of global cognition proximate to death[9] and a slower rate of late-life cognitive decline.[26] Indeed, RAS inhibitor use has been long known to be associated with better cognitive performance and a lower risk of dementia, the clinical manifestations of underlying neurodegenerative and cerebrovascular pathologies in aging.[3]

A few studies have investigated the effect of RAS inhibitors on AD brain pathology, and most of them were conducted with ACE inhibitors in rodent models. One study found increased ACE activity in the brains of rats with AD, and perindopril administration improved learning and memory and delayed amyloid deposition.[29] Another study found that the ACE inhibitor captopril normalized the excessive hippocampal ACE activity and reactive oxygen species, and delayed aging-dependent accumulation of Aβ plaques in the brain of Tg2576 mice.[30] A subsequent study by the same group found that inhibition of ACE using captopril delayed tau hyperphosphorylation and signs of neuronal degeneration in aged rats subjected to chronic unpredictable mild stress.[31] In a recently published study using tau transgenic mice, lisinopril significantly decreased brain levels of total tau and phosphorylated tau-181, and brain levels of lisinopril were negatively correlated with tau.[32] Only one study examined ARBs and AD neuropathology in humans. In this study, participants treated with ARBs showed less amyloid deposition compared to those treated with other antihypertensives and those not taking any hypertensive medications.[33] However, the study was limited by selection biases given that it included only hypertensive participants and excluded cognitively and neuropathologically normal participants. In the present study, using clinical and postmortem neuropathologic data from a group of community-based older individuals across the spectrum of blood pressures and of medication treatments, with and without medical conditions, we provide novel evidence for an association of RAS inhibitor use, mainly driven by ACE inhibitors, with a lower level of tau tangle density. Our finding is in line with a previous human postmortem brain tissue study that directly measured Ang-II and Ang-III (a direct active metabolite of Ang II) levels in 90 patients with Alzheimer's dementia and 59 age-matched nondemented controls.[34] In that study, Kehoe and colleagues found that both Ang-II and Ang-III levels were significantly higher in the Alzheimer's dementia group than in the control group and that Ang-III was strongly associated with amyloid and tau loads.[34] Our study result of an association of RAS inhibitors with lower postmortem tau in the brain is in keeping with this prior

study and expands results to examine inhibition of the RAS system by medications and with more neuropathology outcomes (infarcts and vessel pathologies) while taking into account other vascular conditions (e.g., diabetes).

Because some data suggest a possible association of diabetes to AD pathology and RAS inhibitor users often have comorbid diabetes, we examined in secondary analyses whether the presence of diabetes affected our findings. We found significant interaction effects between diabetes and RAS inhibitors on global AD pathology and specifically on tau tangle density. Furthermore, and consistently, stratified analyses showed that RAS inhibitors were associated with less global AD pathology and less tau tangle density, but only in participants with diabetes and not in those without. These findings may be attributed to diabetes being associated with more AD pathology. In this case, the effect of RAS inhibitors on AD could be limited by relatively low tau accumulation in participants without diabetes. However, we did not find a difference in AD pathology between those with diabetes and those without in our small sample.[9] Another mechanism underlying the favorable effect of ACE inhibitor on AD pathology in participants with diabetes might involve brain insulin signaling, particularly AKT1 phosphorylation. We reported in a previous study using data from the same group of participants that diabetes is associated with a higher level of AKT1 phosphorylation and that AKT1 phosphorylation was positively associated with amyloid burden and tau tangle density.[9] In this study, we found that RAS inhibitor use was no longer statistically significantly related to tau tangle density after including AKT1 in the model, with a reduction of 20.7% in effect size. This suggests that AKT1 phosphorylation might act as a mediator in the association between ACE inhibitor and tau tangle density in older individuals with diabetes. This finding needs to be confirmed in future studies with larger sample sizes and adequate power to detect associations of interest. Interestingly, we did not find an association between ACE inhibitor use and amyloid burden in the postmortem brain of older individuals with or without diabetes. Amyloid is a relatively early marker of AD pathology,[35] and its accumulation is associated with subsequent tau accumulation and then cognitive decline in clinical Alzheimer's disease.[36] Therefore, it is possible that too much amyloid has been accumulated for late-life RAS inhibitor use to take effect. In contrast, all the above-mentioned animal studies examined the effect of ACE inhibitor on amyloid using rodents at an early stage of AD, when amyloid just starts to accumulate, whereas our human study includes persons with more advanced stage AD.

Last, we examined the association of RAS inhibitor use with neuropathologies of cerebrovascular diseases, another important contributor to cognitive impairment in older individuals.[37] We found that RAS inhibitor use was associated with an increased odds ratio of developing moderate to severe atherosclerosis. These findings are largely in line with the results of a previous study also using postmortem human brain tissues, which showed that ARB users had more frequent pathologic evidence of large-vessel infarcts and hemorrhages as well as strokes.[37] The underlying mechanism of these associations might be that individuals with hypertension who need pharmacological treatment such as RAS inhibitors already have higher risks of developing atherosclerosis and vascular diseases including stroke, and not the other way around.[38,39] This speculation that patients with more atherosclerosis are more likely to use RAS inhibitors is also compatible with the findings of previous animal studies suggesting that RAS inhibitors attenuated ischemic brain damage and improved cerebral blood flow.[40,41] After adjusting for vascular risk factors and mean blood pressures, the association was no longer significant, suggesting these factors likely acted as confounders in the association.

Mechanisms by which RAS inhibitors may affect the brain are likely to be both through direct and indirect pathways. While some RAS may cross the blood–brain barrier (BBB) and exert effects directly such as captopril and telmisartan,[42] alternate mechanisms of indirect action are also likely. It is now recognized that BBB disruption is common in both healthy aging itself[43] and in patients with hypertension.[44] Therefore, it is possible that RAS inhibitors can have a direct effect on the brain molecular pathways in older adults with and without hypertension. In addition, relevant to ACE inhibitors specifically, circulating Ang II can gain access to the brain via the breakdown of the BBB in a hypertensive mouse model.[45] Even in physiological states, Ang II has been shown to cross brain microvessel endothelial cells via AT1 receptor-mediated transcytosis.[46] Therefore, ACE inhibitors, which make up the majority of RAS inhibitors in our study, might be able to indirectly impact brain insulin signaling by decreasing the level of circulating Ang II. More research is warranted to understand these and other mechanisms underlying the association between RAS inhibitor use and brain insulin signaling. While we considered analyses breaking down RAS inhibitors by their BBB-crossing properties, the sample sizes would be prohibitive.

This study has several limitations. First, the study participants were predominantly non-Hispanic white, community-dwelling Catholic clergy who agreed to annual follow-up visits and brain autopsy after death, and they might not be representative of the general population of older adults. Second, the sample size was relatively small, which could have limited the statistical power of our analyses to detect some important associations and led to Type-II errors. Future studies are warranted to

reproduce our findings with larger, independent samples. Third, the effects of RAS inhibitor use in our study were largely attributed to ACE inhibitor use. Due to the small percentage of ARB users and the fact that half of the ARB users also used ACE inhibitors, we were unable to determine the effect of "pure ARB use" on brain insulin signaling and neuropathology. Likewise, given that the vast majority of RAS inhibitor users in our study also had used other classes of antihypertensives concomitantly, we could not exclude the possibility that other antihypertensives, such as diuretics or beta-blockers, acted as confounders. And while we controlled for vascular risk factors including diabetes, hypertension and smoking, as well as for systolic and diastolic blood pressures, we cannot exclude the possibility of confounding by indication. Fourth, data on total exposure time and cumulative dosing of RAS inhibitors were not available (including before baseline), and we were unable to determine their effects on the associations we examined in this study. Last, we only measured IRS-1 and AKT-1 in the postmortem prefrontal cortex. Our findings might not apply to other brain regions, and furthermore, RAS inhibitor use could be associated with other brain insulin signaling molecules. Nonetheless, this study has several notable strengths. First and foremost, we studied persons with and without diabetes from a large cohort study with a high autopsy rate using a rigorous nested case–control design. In addition, our medication use data were gathered through direct visual inspection of medication containers during each study visit, as opposed to relying on patient-reported information which can be susceptible to recall bias. Also, we studied the associations of late-life RAS inhibitors with postmortem brain insulin signaling molecules measured using two complementary approaches, which offer new and valuable insights into the interaction between RAS and brain insulin signaling pathways. Last but not least, our systemically collected and well-characterized neuropathology data encompass common pathologies associated with dementia, including Alzheimer's disease, cerebral infarcts, and cerebral vessel diseases.

Author Contributions

H.T., A.W.C., D.A.B., and Z.A. contributed to the conception and design of the study; H.T., A.W.C., R.I.M., D.A.B., S.E.A., R.S.A., and Z.A. contributed to the acquisition and analysis of data; H.T., A.S., and Z.A. contributed to drafting the text or preparing the figures.

Acknowledgments

The authors sincerely thank all participants in the Religious Orders Study for their altruism and commitment to this ongoing study since 1994. The authors also thank all Rush Alzheimer's Disease Center staff and faculty, in particular, Traci Colvin for study coordination, Ryan Johnson for laboratory management, John Gibbons for data management, and Alysha Hodges for statistical programming.

Funding Information

This work was funded by the NIH grants: P30AG10161, P30AG072975, R01AG15819, R01NS084965, RF1AG059621, and RF1AG074549.

Conflict of Interest

The authors have no conflicts of interest to declare.

Data Availability Statement

All data supporting the conclusions of this article can be requested via the Rush Alzheimer's Disease Center Research Resource Sharing Hub at www.radc.rush.edu.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1.

