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Letter

Rhynchophylline Administration Ameliorates Amyloid-β Pathology and Inflammation in an Alzheimer's Disease Transgenic Mouse Model

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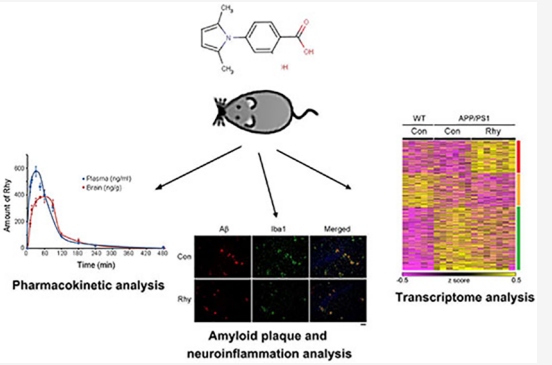
ABSTRACT: Alzheimer's disease (AD), the most common neurodegenerative disease, has limited treatment options. As such, extensive studies have been conducted to identify novel therapeutic approaches. We previously reported that rhynchophylline (Rhy), a small molecule EphA4 inhibitor, rescues impaired hippocampal synaptic plasticity and cognitive dysfunctions in APP/PS1 mice, an AD transgenic mouse model. To assess whether Rhy can be developed as an alternative treatment for AD, it is important to examine its pharmacokinetics and effects on other disease-associated pathologies. Here, we show that Rhy ameliorates amyloid plaque burden and reduces inflammation in APP/PS1 mice. Transcriptome analysis revealed that Rhy regulates various molecular pathways in APP/PS1 mouse brains associated with amyloid metabolism and inflammation, specifically the ubiquitin proteasome system, angiogenesis, and microglial functional states. These results show that Rhy, which is blood–brain barrier permeable, is beneficial to amyloid pathology and regulates multiple molecular pathways.



KEYWORDS: *Amyloid, neuroinflammation, microglia, EphA4, ubiquitin proteasome system, angiogenesis*

INTRODUCTION

Alzheimer's disease (AD), the most common type of dementia in the elderly population, is characterized by a deterioration of cognitive performance including learning and memory formation as well as behavioral changes. The major neuropathological changes in AD are the formation of neuritic plaques (i.e., the extracellular aggregation of amyloid-beta [Aβ] peptides) and neurofibrillary tangles (i.e., the intracellular accumulation of hyperphosphorylated tau protein in neurons) along with progressive neuronal loss and brain atrophy.[1] Most existing drugs for AD merely alleviate symptoms and are not effective in all patients. The challenges in developing AD therapies are due to the multifactorial nature of the disease and our incomplete understanding of its underlying pathological mechanisms. The initiation and progression of AD pathogenesis includes various pathophysiological events such as aberrant neuronal activity, dysfunction of the neurovascular unit, and perturbation of immune homeostasis in the brain.[1,2] The perturbation of one of these processes may in turn dysregulate the others during AD progression. Therefore, it may be invaluable to identify a therapeutic approach for AD that has beneficial effects on multiple biological processes.

Rhynchophylline (Rhy), a small molecule isolated from a traditional Chinese medicinal herb, *Uncaria rhynchophylla*, is reported to have beneficial effects in various neurological disorders including dementia, antiepileptic and antidepressant effects, and neuroprotective effects in cerebral ischemia.[3] We previously reported that administration of Rhy rescues the impairment of hippocampal synaptic plasticity, a major cellular mechanism underlying learning and memory, in APP/PS1 mice, a transgenic mouse model of AD.[4] In addition, an isomer of Rhy, isorhynchophylline, modulates the amyloid pathology in TgCRND8 mice, another AD transgenic mouse model.[5] It has been suggested that Rhy may exert its beneficial actions through the inhibition of the receptor tyrosine kinase EphA4 or blockade of NMDA receptors and Ca^{2+} channels;[4,6] EphA4, NMDA receptors, and Ca^{2+} channels play critical roles in AD pathogenesis through modulating distinct biological processes including synaptic functions, neurovascular functions, neuroinflammation, and neuronal survival. Nevertheless, it is unclear

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whether Rhy exerts its beneficial effects in AD through the modulation of multiple biological processes.

In this study, we examined the roles of Rhy in Aβ pathology and microglial activation. Using transcriptome analysis, we also investigated how this small molecule modulates different molecular and cellular pathways in the cerebral cortices of APP/PS1 transgenic mice. Our findings provide insights into the feasibility of developing Rhy as a therapeutic intervention for AD and its underlying mechanisms.

RESULTS AND DISCUSSION

We previously demonstrated that oral administration of Rhy rescues hippocampal synaptic plasticity in APP/PS1 mice.[4] To examine the brain access of Rhy, we determined the pharmacokinetics of Rhy in mouse brains. Table 1 lists the pharmacokinetic parameters of Rhy in mouse plasma and brain after oral administration of Rhy at 50 mg/kg. Rhy was readily detected in both the plasma and brains of adult mice at 10 min after oral administration (Figure 1). The maximum concentration (C_{max}) of Rhy detected in the plasma was 583.20 ± 32.86 ng/mL at 30.84 ± 2.52 min, while C_{max} of Rhy detected in the brain was 442.73 ± 27.38 ng/g at 55.02 ± 9.18 min after oral administration (Figure 1 and Table 1). After ~3 h of administration, the level of Rhy dropped to 10% of C_{max} in both the plasma and brain. These findings demonstrate that Rhy administered orally in mice can rapidly enter the systemic circulation and pass through the blood–brain barrier (BBB) into the brain.

Table 1. Summary of Pharmacokinetic Parameters of Rhynchophylline[a]

	brain	plasma
C_{max}	442.73 ± 27.38 ng/g	583.20 ± 32.86 ng/mL
T_{max}	55.02 ± 9.18 min	30.84 ± 2.52 min
$t_{1/2}$	107.22 ± 27.30 min	81.12 ± 12.06 min

[a] C_{max}, maximum concentration; T_{max}, time it takes to achieve C_{max}; $t_{1/2}$, elimination half-life; for rhynchophylline in mouse brain and plasma.

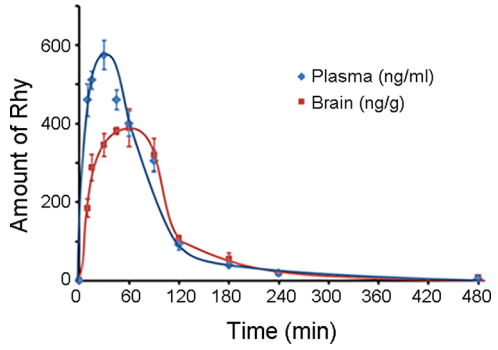

Figure 1. Pharmacokinetics of rhynchophylline in mouse plasma and brain after oral administration. Data shown are the amount of rhynchophylline (Rhy) in mouse plasma (ng/mL) or brain (ng/g) after administration (mean ± SEM). Blood and perfused whole brains were collected at different time points after a single administration of Rhy. N = 6 animals per time point.

After determining the bioavailability of Rhy in the mouse brains, we examined whether Rhy administration exerts beneficial effects on other AD-related pathologies, in addition to the alleviation of synaptic plasticity dysfunction. Accordingly, we examined the amyloid plaque burden in 6 to 7 month old APP/PS1 mice after oral administration of Rhy for at least 4 weeks. In APP/PS1 mice treated with the vehicle, we observed amyloid plaque accumulation in the cerebral cortex and hippocampus, whereas Rhy administration significantly decreased the total area of amyloid plaques in both brain regions (Figure 2A,B). Moreover, we examined the levels of soluble and insoluble Aβ in the cerebral cortex in APP/PS1 mice. Soluble Aβ oligomers are suggested as a causative factor for synaptotoxicity in AD.[7] Concordantly, Western blot analysis showed that Rhy administration significantly reduced the soluble fractions (i.e., DEA-extracted fractions) of Aβ in the cerebral cortices of APP/PS1 mice (Figure 2C,D), whereas the Aβ content in insoluble fractions (i.e., FA-extracted fractions) remained relatively unchanged (Figure 2C,E). In particular, the levels of $A\beta_{x-40}$ and $A\beta_{x-42}$ in the soluble fractions of Rhy-treated transgenic mice cortices were ~30% and ~20% lower than those in the vehicle-treated condition, respectively (Figure 2F,G).

The dysregulation of immune homeostasis and inflammation, critical etiological factors in AD, is highly associated with the extent of amyloid plaque deposition.[8] Microglia, the key immune cell type in the brain, are found in the vicinity of amyloid plaques and subsequently clear them. Excessive activation of microglia results in increased production of inflammatory cytokines, resulting in neuroinflammation.[9] While Iba1 staining revealed extensive plaque-associated microgliosis in the cortices of APP/PS1 mice, Rhy administration significantly decreased the number of plaque-associated reactive microglia in APP/PS1 mice (Figure 3A–D). Consistent with this decrease in microglial activation, Rhy administration also resulted in decreased transcript levels of various inflammation-associated genes, namely *SPP1*, *GPNMB*, and *ITGAX* (Figure 3E–G). The *SPP1* gene encodes the protein "osteopontin", which is upregulated in AD plasma.[10] The *GPNMB* gene encodes a glycoprotein named "osteoactivin" that is highly expressed in microglia. These 2 proteins play roles in anti-inflammation and tissue repair.[11−13] The *ITGAX* gene encodes a protein called "integrin, alpha X," also known as "CD11c," which is suggested to mediate inflammatory responses.[14] Notably, these three genes are enriched in activated response microglia (ARMs), a specific microglial subtype induced in a transgenic AD mouse model.[11] A decrease of these genes in Rhy-treated APP/PS1 mice suggests that Rhy reduces the inflammatory status and regulates microglial functional states in APP/PS1 mouse brains. These results collectively show that besides rescuing hippocampal synaptic impairment in APP/PS1 mice,[4] Rhy ameliorates amyloid pathology and modulates inflammation in APP/PS1 mice.

To study how Rhy ameliorates amyloid pathology, we examined whether Rhy affects the generation of Aβ peptides. Aβ peptides are generated through the proteolytic processing of APP (amyloid precursor protein) by three proteases: α-, β-, and γ-secretase. APP can be cleaved by α- or β- secretase, yielding the c-terminal fragments of APP (APP-CTFs), C83 and C99, respectively.[15] Further cleavage of C99 by γ-secretase generates Aβ peptides. Interestingly, we found that APP-CTFs were lowered in the brains of Rhy-treated APP/PS1 mice (Figure 4). Meanwhile, Rhy administration did not affect APP expression levels (Figure 4). These results suggest that Rhy may regulate APP processing in different ways: i.e., Rhy may

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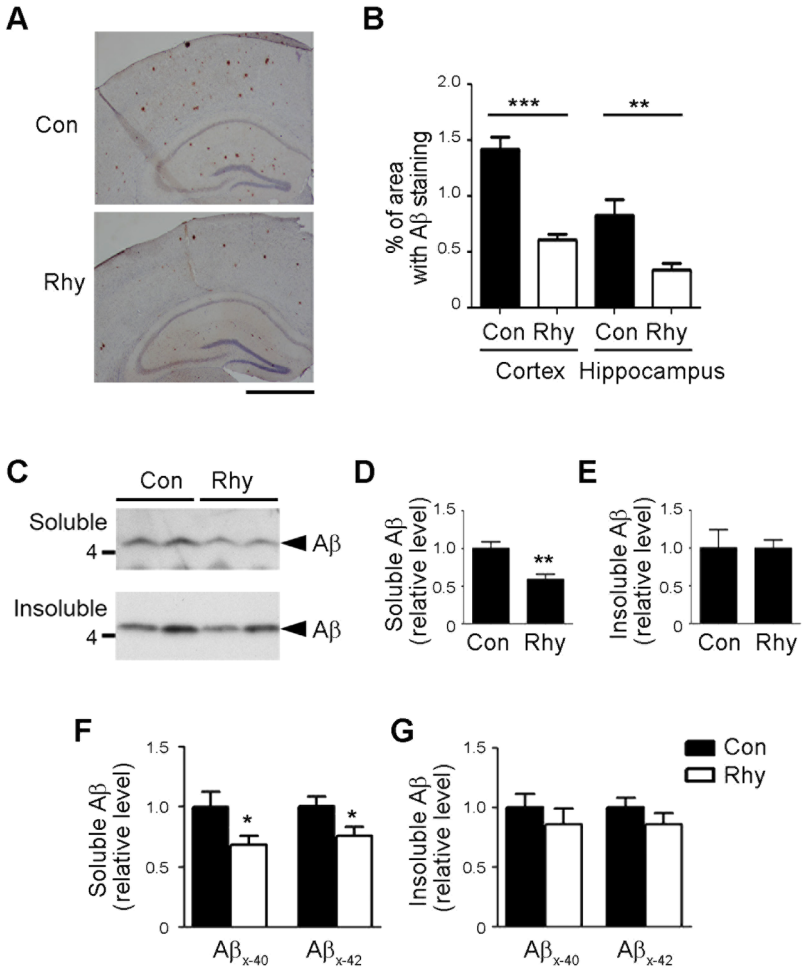

Figure 2. Rhynchophylline ameliorates amyloid pathology in APP/PS1 transgenic mice. APP/PS1 transgenic mice at 6−7 months of age were administered with rhynchophylline (Rhy) (50 mg·kg^{-1}·day^{-1}) for at least 4 weeks. (A,B) Rhy administration leads to decreased amyloid plaque deposition in APP/PS1 mice. (A) Representative images. Scale bar = 1 mm. (B) Quantification of the area covered by amyloid plaque deposition in the cortex and hippocampus (>4 sections from each mouse brain, n = 4 brains). Data are presented as mean ± SEM, ***p < 0.001, **p < 0.01, Student's t-test. (C−G) Rhy reduces soluble amyloid-beta ($A\beta$) levels in APP/PS1 mice. (C−E) Western blot analysis of $A\beta$ levels in the soluble and insoluble fractions of the cerebral cortex in APP/PS1 mice. Representative blots (C) and quantitative analysis of soluble (D) and insoluble (E) $A\beta$ fractions. Each lane represents an individual mouse (n = 6 mice for Con, n = 9 mice for Rhy). (F,G) Quantitative analysis of $A\beta_{x-40}$ and $A\beta_{x-42}$ levels in soluble (F) and insoluble (G) fractions of cortex homogenates using ELISA (fold change compared to Con [vehicle control]; n = 7 mice for Con, 11 mice for Rhy).

directly affect the generation of APP-CTFs through regulating the activities of α- or β-secretase,[15] or it may prevent the accumulation of APP-CTFs through regulating the expression of secretases or APP-CTFs by autophagosomal lysosomal pathway and endoplasmic reticulum-associated degradation.[16,17] Moreover, Rhy may regulate γ-secretase activity that facilitates the clearance of APP-CTFs.[15]

As Rhy can regulate multiple molecular targets, it is of interest to examine how Rhy exhibits its beneficial effects in AD through its regulation of multiple biological pathways. As such, we conducted a transcriptome analysis on the cerebral cortices of APP/PS1 mice after Rhy administration. Hierarchical clustering of the differentially expressed genes revealed that three gene modules were differentially regulated among wild-type (WT) mice, vehicle-treated APP/PS1 mice, and Rhy-treated APP/PS1 mice (Figure 5A). Regarding the first gene module, while there were no obvious changes in gene expression between WT and vehicle-treated APP/PS1 mice, Rhy administration increased the expression level of these genes (Figure 5A). The second gene module included genes that were downregulated in APP/PS1 mice when compared to WT mice; these genes were further downregulated in Rhy-treated APP/PS1 mice compared to those in vehicle-treated APP/PS1 mice. Meanwhile, the genes in the third gene module were upregulated in APP/PS1 mice compared to WT mice; the upregulation of these genes was partially restored in Rhy-treated APP/PS1 mice (Figure 5A).

Regarding the first module of genes, which were upregulated in the brains of Rhy-treated APP/PS1 mice, Gene Ontology (GO) and protein−protein interaction (PPI) analyses showed that these genes are associated with microtubule-based processes, intracellular transport, cell differentiation, and proteolysis (Figure 5B,C). Proteolysis, a process that includes the ubiquitin proteasome system (UPS) and autophagosomal lysosomal pathway (ALP), is critical for the protein quality control via removal of misfolded proteins or aggregates, and hence the maintenance of neural cell functioning.[17,18] Both the UPS and ALP regulate APP processing in neurons, which results in the regulation of $A\beta$ secretion, aggregation, and degradation; thus, the dysregulation of these processes in

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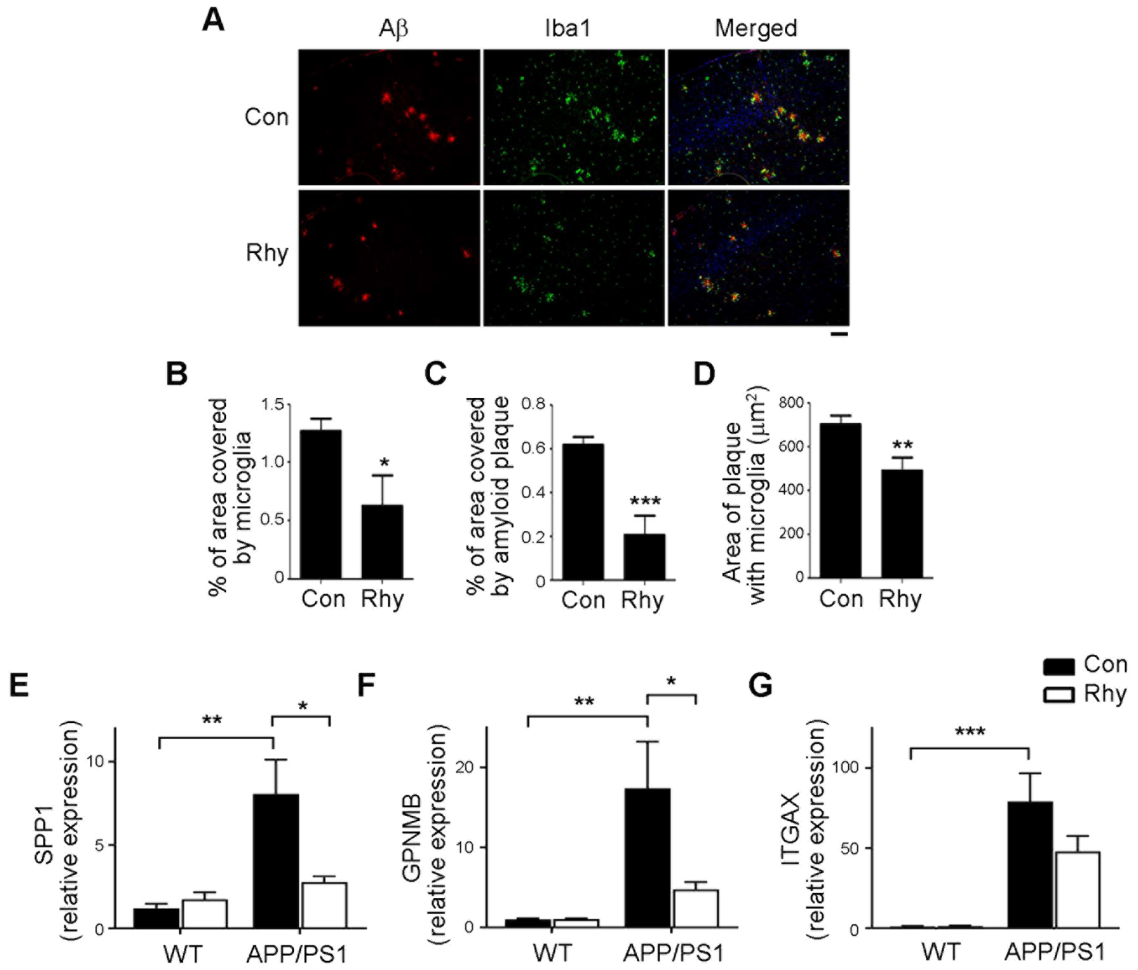

Figure 3. Rhynchophylline administration leads to amelioration of neuroinflammation in APP/PS1 mice. APP/PS1 mice at 6−7 months of age were administered with rhynchophylline (Rhy) (50 $mg \cdot kg^{-1} \cdot day^{-1}$) for at least 4 weeks. (A−D) Brain sections of APP/PS1 mice were co-immunostained with antibodies against amyloid-beta (Aβ) (red; amyloid plaque deposition) and Iba-1 (green; microglia). (A) Representative images. Scale bar = 100 μm. Quantification analysis of percentage of area covered by microglia (B) and amyloid plaque (C), and area (D) of plaque-associated microglia (two sections from each mouse brain, $n = 3$ brains). Data are presented as mean ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, Student's t-test. (E−G) Rhy administration modulates inflammatory responses in APP/PS1 transgenic mice. Quantitative PCR analysis showing the regulation of inflammatory genes in the cerebral cortex in wild-type (WT) and APP/PS1 mice. All measurements are presented as the expression fold change relative to the average of the WT-Con group. Transcript levels of *SPP1* (E), *GPNMB* (F), and *ITGAX* (G) (WT-Con, $n = 5$; WT-Rhy, $n = 5$; APP/PS1-Con, $n = 6$; App/PS1-Rhy, $n = 7$). All data are mean ± SEM ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; two-way ANOVA with Tukey's post hoc test). Con, vehicle control.

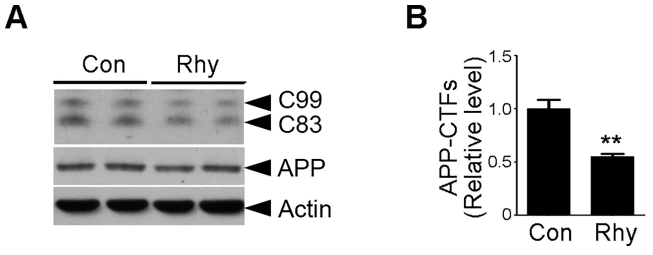

Figure 4. Amount of APP c-terminal fragments decreases in rhynchophylline-treated APP/PS1 mouse brains. (A) Total mouse brain lysate was subjected to Western blot analysis for APP-CTFs (C99 and C83) and APP (actin was used as a loading control). (B) Quantification analysis of APP-CTFs. Data are presented as mean ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$, Student's t-test. APP, amyloid precursor protein; APP-CTFs, APP c-terminal fragments; Con, vehicle control; Rhy, Rhynchophylline.

neural cells may contribute to AD pathology.[17,18] In glial cells, the UPS is also critical for degrading pathological proteins (e.g., Aβ peptides) and mediating neuroinflammation.[19] PPI analysis revealed that most of the genes are components of the E3 ubiquitin ligase complex in the UPS (e.g., *CUL9*, *FBXL2*, *FBXL13*, *LNX1*, *MAEA*, *RNF114*, *SIAH3*, *TRIM32*, *TRIM63*, *UBE3A*, *UBR1*, and *UBR4*) or peptidases (e.g., *AGTPBP1*, *ADAMTS3*, *ADAMTS10*, *ADAMTS20*, *DPP6*, *MMP7*, *MMP12*, *MMP16*, *USP2*, *USP4*, *USP15*, *USP18*, *USP24*, *USP36*, and *USP45*). Several upregulated genes (e.g., *UBE2J1*, *ERLEC1*, *HERPUD1*, and *UBXN4*) are associated with the endoplasmic reticulum-associated protein degradation pathway. Moreover, some regulated genes are associated with autophagy regulation (e.g., *BAG6*, *CTSK*, *CYLD*, *HDAC6*, *HERPUD1*, *SIRT2*, *TRIM32*, *UBE4B*, *UBR4*, and *USP18*). The present study shows that Rhy administration increases the proteolytic activity in APP/PS1 mouse brains, which provides insights into the underlying mechanisms of how Rhy decreases soluble Aβ levels and amyloid plaque deposition.

Meanwhile, the genes in the second module are associated with epithelial morphogenesis, epithelial tube morphogenesis,

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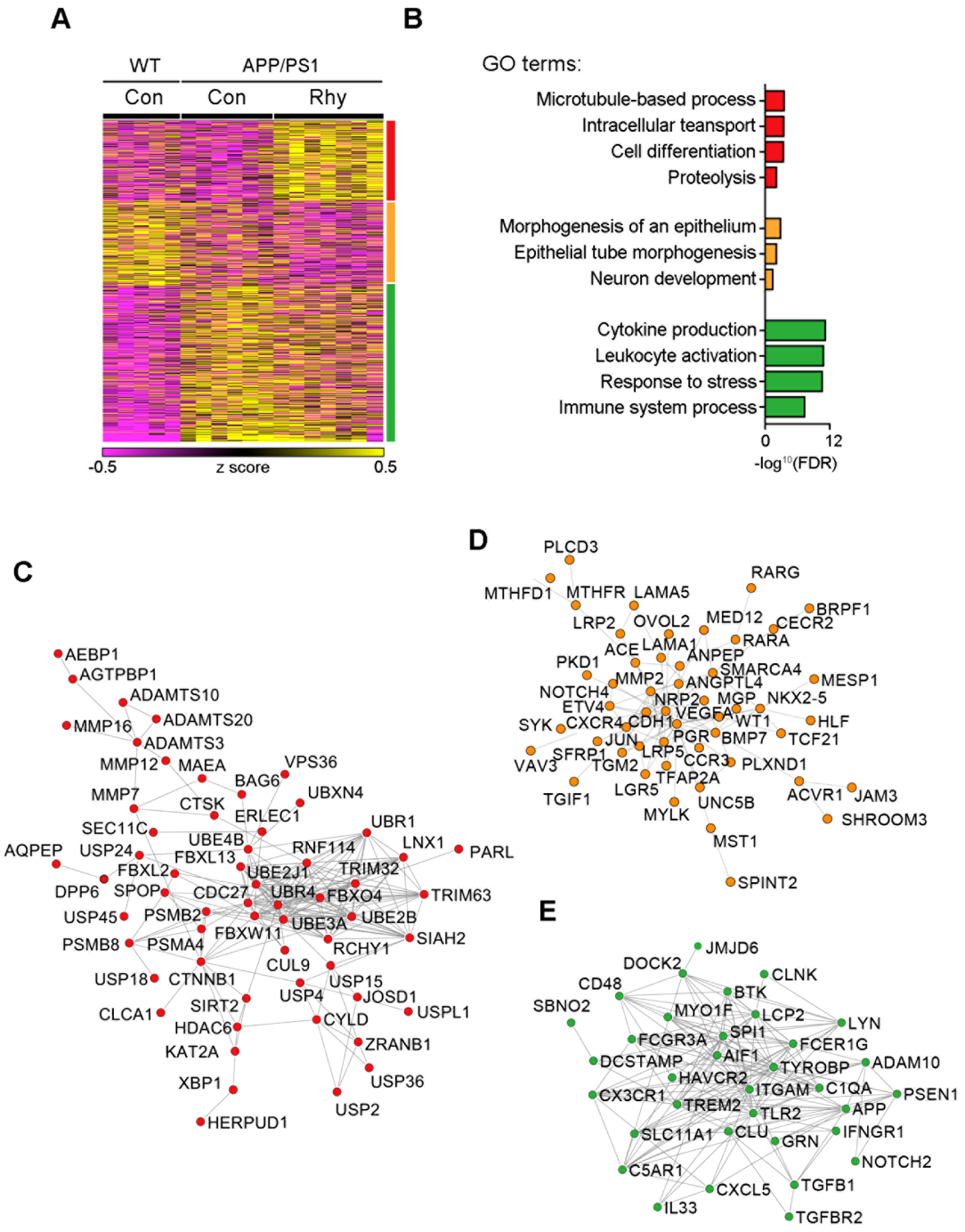

Figure 5. Administration of rhynchophylline regulates the transcriptomic signature in the cerebral cortex in APP/PS1 mice. (A) Heat map showing the relative expression levels of differentially expressed genes (DEGs) in Rhy- versus Con-treated APP/PS1 mice and Con-treated WT mice. (B) Gene Ontology (GO) pathway analysis of the DEGs. (C−E) STRING protein−protein interaction analysis of the DEGs. All data are mean ± SEM. Con, vehicle control; FDR, false discovery rate; Rhy, rhynchophylline; WT, wild-type.

and neuronal development (Figure 5B,D). Many genes in this module are key signaling molecules that regulate angiogenesis and affect the vascular network (e.g., *ANGPTL4*, *ANPEP*, *BMP7*, *CXCR4*, *MMP2*, *MST1*, *NOTCH4*, *NRP2*, *PLXND1*, *UNC5B*, and *VEGFA*) and functions related to blood flow and BBB permeability (e.g., *ACE*, *CDH1*, *JAM3*, *LRP2*, *MTHFR*, *SMARCA4*, and *SYK*). Dysfunctions of the neurovascular system and disruption of the BBB contribute to AD pathology;[20,21] these interfere with the Aβ efflux from the brain and allow unwanted molecules or immune cells to enter the brain,[20,22] which contributes to amyloid plaque deposition, neuroinflammation, and neuronal dysfunction in AD.[20,21] Previous findings suggest that Rhy protects BBB integrity in rats with subarachnoid hemorrhage.[23] Thus, our results provide additional molecular insights on how Rhy modulates the functions and/or stability of the neurovascular system, thereby contributing to the amelioration of amyloid pathology.

Regarding the third gene module, PPI analysis revealed that these genes are involved in cytokine production, leukocyte activation, stress response, and immune system processes. Interestingly, most of these regulated genes are microglial genes (e.g., *AIF1*, *C1QA*, *C5AR1*, *CX3CR1*, *DOCK2*, *MYO1F*,

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SPI1, *ITGAM*, *TGFB1*, *TGFBR2*, *TLR2*, *TREM2*, and *TYROBP*) (Figure 5E).[24] Microglia, the resident immune cells in the brain, are critical for maintaining central nervous system homeostasis. Microglia continuously sense and change their functional states in response to the environment. Different subtypes of microglia with unique signatures and functional properties are present during different developmental stages as well as in various neurodegenerative diseases and disorders.[11,24,25] For example, disease-associated microglia, microglial neurodegenerative phenotype and ARM are identified to be induced in response to Aβ accumulation in various AD mouse models.[11,26,27] Accordingly, our bulk transcriptome analysis revealed that the signature genes of both homeostatic microglia (e.g., *CX3CR1*, *TGFB1*, and *TGFBR2*) and disease-associated microglia (e.g., *TREM2* and *TYROBP*) are modulated in Rhy-treated APP/PS1 mice (Figure 5E), suggesting that Rhy may modulate the functional state transition of microglia in AD.[26] Notably, activation of IL-33 signaling and PU.1 (encoded by *IL33* and *SPI1* in this gene cluster, respectively; Figure 5E) induces the state transition of a microglial subpopulation toward enhanced phagocytic capacity and Aβ clearance, thereby ameliorating amyloid plaque deposition.[28,29] Nevertheless, further studies, such as single-cell RNA sequencing analyses, are required to better understand the beneficial effects of Rhy on regulating microglial functional state activation and how the transition of microglia may contribute to the amelioration of neuroinflammation in AD.

Our previous study and the present findings indicate that Rhy ameliorates AD pathology, including the alleviation of synaptic plasticity impairment and amyloid plaque burden.[4,5,30,31] Here, we also investigate the molecular mechanisms underlying the beneficial effects of Rhy in AD. While Rhy is suggested to be an NMDA antagonist and calcium channel blocker,[6] we showed that Rhy targets EphA4 through its ligand-binding domain and that Rhy attenuates Aβ-stimulated aberrant EphA4 activation in APP/PS1 mice.[4] EphA4, a member of the Eph (erythropoietin-producing hepatocellular) family of receptor tyrosine kinases, together with its cognate ligands (i.e., ephrins) signals bidirectionally; this signaling is important for regulating various cellular processes of the central nervous system, including synaptic plasticity, neuroinflammation, and vascular permeability.[32] A recent genetics study identified a single-nucleotide polymorphism proximal to the *EPHA4* gene that is associated with AD.[33] Neural cells near amyloid plaques and neurofibrillary tangles in patients with AD exhibit increased expression of the EphA4 protein.[34] Besides their impacts on synaptic plasticity, EphA4 and ephrins mediate microglial proliferation, microglial phenotype transition, and inflammatory response modulation via signaling between neurons and microglia.[35] Thus, Rhy may alleviate AD pathology by modulating inflammatory and immune responses in the brain. It is of interest to examine whether Rhy exerts its beneficial effects in AD through EphA4 signaling.

In conclusion, Rhy administration alleviates synaptic plasticity defects, amyloid plaque burden, and neuroinflammation in AD transgenic model mice. Transcriptome analysis reveals that Rhy targets specific molecular and cellular processes, which may contribute to the alleviation of AD pathologies. Together, these findings suggest that small molecule Rhy has the potential to be developed as a therapeutic intervention for AD.

METHODS

Chemicals and Antibodies. The following antibodies were used: anti-Aβ (6F/3D, Dako, Denmark; and 6E10, BioLegend, USA); anti-Iba-1 (Wako Pure Chemical Industries, USA); and anti-actin (A3853) and anti-APP (A8717) from Sigma-Aldrich, USA. Rhy was from Baoji Herbest Bio-Tech, China.

Animals and Drug Administration. APP/PS1 double-transgenic mice (B6C3-Tg[APPswe, PSEN 1dE9]85Dbo/J) were obtained from Jackson Laboratory, USA. The genotype was confirmed by PCR analysis of tail biopsies. Four to five mice of the same sex were housed per cage on a 12 h light/dark cycle with food and water ad libitum. Mice were orally administered with Rhy 50 $mg \cdot kg^{-1} \cdot day^{-1}$ by daily gavage.

Detection of Rhynchophylline in Blood Plasma and Brain. The Supporting Information describes Rhy bioavailability analysis, preparation of mouse blood plasma and brain tissues, and details regarding the bioavailability determination of Rhy.

Amyloid-β Extraction and ELISA. Aβ was sequentially extracted from soluble and insoluble fractions in mouse brains as previously described.[29] $A\beta_{X\text{-}40}$ and $A\beta_{X\text{-}42}$ were analyzed by either Western blot analysis or ELISA (Thermo Fisher Scientific, USA).

Immunohistochemical, Confocal Microscopy, and Quantitative Analyses. The Supporting Information outlines the details regarding the immunohistochemical and quantitative analyses.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acschemneuro.1c00600.

Additional materials and methods; preparation of blood plasma and brain tissues for bioavailability determination of Rhy; methods for detection of Rhy in blood plasma and brain; methods for immunohistochemical, confocal microscopy, and quantitative analyses; RNA extraction for microarray transcriptome analysis; real time-qPCR analysis; microarray transcriptome analysis and statistical analysis; table of the primer sequences for real-time qPCR (PDF)

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Author Contributions

W−Y.F., A.K.F., and N.Y.I. conceived the study. W.-Y.F., F.C.I., A.K.F., and N.Y.I. designed the experiments. W.-Y.F., K.-W.H., G.F., B.B., V.W.Y., and I.C.C. conducted the experiments. W.-Y.F., K.-W.H., S.-F.L., B.B., V.W.Y., I.C.C, A.K.F., and N.Y.I. analyzed the data. W.-Y.F., A.K.F., and N.Y.I. wrote the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

Aβ, amyloid-beta; AD, Alzheimer's disease; APP, amyloid precursor protein; APP-CTFs, c-terminal fragments of APP; BBB, blood−brain barrier; $Ca2^{+}$, calcium ion; C_{max}, maximum concentration; Con, vehicle control; DEGs, differentially expressed genes; Eph, erythropoietin-producing hepatocellular; FDR, false discovery rate; GO, Gene Ontology; NMDA, *N*-methyl-D-aspartate; PPI, protein−protein interaction; Rhy, Rhynchophylline; $t_{1/2}$, elimination half-life; T_{max}, the time it takes to achieve C_{max}; UPS, ubiquitin proteasome system; WT, wild-type

■ REFERENCES

(1) Long, J. M.; Holtzman, D. M. Alzheimer Disease: An Update on Pathobiology and Treatment Strategies. *Cell* **2019**, *179*, 312−339.

(2) Fu, W. Y.; Wang, X.; Ip, N. Y. Targeting Neuroinflammation as a Therapeutic Strategy for Alzheimer's Disease: Mechanisms, Drug Candidates, and New Opportunities. *ACS Chem. Neurosci.* **2019**, *10*, 872.

(3) Shi, J. S.; Yu, J. X.; Chen, X. P.; Xu, R. X. Pharmacological Actions of Uncaria Alkaloids, Rhynchophylline and Isorhynchophylline. *Acta Pharmacol. Sin.* **2003**, *24* (2), 97−101.

(4) Fu, A. K. Y.; Hung, K. W.; Huang, H.; Gu, S.; Shen, Y.; Cheng, E. Y. L.; Ip, F. C. F.; Huang, X.; Fu, W. Y.; Ip, N. Y. Blockade of EphA4 Signaling Ameliorates Hippocampal Synaptic Dysfunctions in Mouse Models of Alzheimer's Disease. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (27), 9959−9964.

(5) Li, H. Q.; Ip, S. P.; Yuan, Q. J.; Zheng, G. Q.; Tsim, K. K. W.; Dong, T. T. X.; Lin, G.; Han, Y.; Liu, Y.; Xian, Y. F.; Lin, Z. X. Isorhynchophylline Ameliorates Cognitive Impairment via Modulating Amyloid Pathology, Tau Hyperphosphorylation and Neuroinflammation: Studies in a Transgenic Mouse Model of Alzheimer's Disease. *Brain, Behav., Immun.* **2019**, *82*, 264−278.

(6) Zhou, J.; Zhou, S. Antihypertensive and Neuroprotective Activities of Rhynchophylline: The Role of Rhynchophylline in Neurotransmission and Ion Channel Activity. *J. Ethnopharmacol.* **2010**, *132* (1), 15−27.

(7) Chen, Y.; Fu, A. K. Y.; Ip, N. Y. Synaptic Dysfunction in Alzheimer's Disease: Mechanisms and Therapeutic Strategies. *Pharmacol. Ther.* **2019**, *195* (xxxx), 186−198.

(8) Heneka, M. T.; Golenbock, D. T.; Latz, E. Innate Immunity in Alzheimer's Disease. *Nat. Immunol.* **2015**, *16*, 229.

(9) Cameron, B.; Landreth, G. E. Inflammation, Microglia, and Alzheimer's Disease. *Neurobiol. Dis.* **2010**, *37* (3), 503−509.

(10) Chai, Y. L.; Chong, J. R.; Raquib, A. R.; Xu, X.; Hilal, S.; Venketasubramanian, N.; Tan, B. Y.; Kumar, A. P.; Sethi, G.; Chen, C. P.; Lai, M. K. P. Plasma Osteopontin as a Biomarker of Alzheimer's Disease and Vascular Cognitive Impairment. *Sci. Rep.* **2021**, *11* (1), 1−11.

(11) Sala Frigerio, C.; Wolfs, L.; Fattorelli, N.; Thrupp, N.; Voytyuk, I.; Schmidt, I.; Mancuso, R.; Chen, W. T.; Woodbury, M. E.; Srivastava, G.; Möller, T.; Hudry, E.; Das, S.; Saido, T.; Karran, E.; Hyman, B.; Perry, V. H.; Fiers, M.; De Strooper, B. The Major Risk Factors for Alzheimer's Disease: Age, Sex, and Genes Modulate the Microglia Response to Aβ Plaques. *Cell Rep.* **2019**, *27* (4), 1293−1306.e6.

(12) Yu, B.; Alboslemy, T.; Safadi, F.; Kim, M. H. Glycoprotein Nonmelanoma Clone B Regulates the Crosstalk between Macro-

phages and Mesenchymal Stem Cells toward Wound Repair. *J. Invest. Dermatol.* **2018**, *138* (1), 219−227.
(13) Icer, M. A.; Gezmen-Karadag, M. The Multiple Functions and Mechanisms of Osteopontin. *Clin. Biochem.* **2018**, *59* (July), 17−24.
(14) Karnell, J. L.; Kumar, V.; Wang, J.; Wang, S.; Voynova, E.; Ettinger, R. Role of CD11c+ T-Bet+ B Cells in Human Health and Disease. *Cell. Immunol.* **2017**, *321* (July), 40−45.
(15) Haass, C.; Kaether, C.; Thinakaran, G.; Sisodia, S. Trafficking and Proteolytic Processing of APP. *Cold Spring Harbor Perspect. Med.* **2012**, *2* (5), a006270.
(16) González, A. E.; Muñoz, V. C.; Cavieres, V. A.; Bustamante, H. A.; Cornejo, V. H.; Januário, Y. C.; González, I.; Hetz, C.; Dasilva, L. L.; Rojas-Fernández, A.; Hay, R. T.; Mardones, G. A.; Burgos, P. V. Autophagosomes Cooperate in the Degradation of Intracellular C-Terminal Fragments of the Amyloid Precursor Protein via the MVB/Lysosomal Pathway. *FASEB J.* **2017**, *31* (6), 2446−2459.
(17) Aman, Y.; Schmauck-Medina, T.; Hansen, M.; Morimoto, R. I.; Simon, A. K.; Bjedov, I.; Palikaras, K.; Simonsen, A.; Johansen, T.; Tavernarakis, N.; Rubinsztein, D. C.; Partridge, L.; Kroemer, G.; Labbadia, J.; Fang, E. F. Autophagy in Healthy Aging and Disease. *Nat. Aging* **2021**, *1* (8), 634−650.
(18) Bustamante, H. A.; González, A. E.; Cerda-Troncoso, C.; Shaughnessy, R.; Otth, C.; Soza, A.; Burgos, P. V. Interplay between the Autophagy-Lysosomal Pathway and the Ubiquitin-Proteasome System: A Target for Therapeutic Development in Alzheimer's Disease. *Front. Cell. Neurosci.* **2018**, *12* (May), 1−18.
(19) Heneka, M. T.; Carson, M. J.; Khoury, J. El; Landreth, G. E.; Brosseron, F.; Feinstein, D. L.; Jacobs, A. H.; Wyss-Coray, T.; Vitorica, J.; Ransohoff, R. M.; Herrup, K.; Frautschy, S. A.; Finsen, B.; Brown, G. C.; Verkhratsky, A.; Yamanaka, K.; Koistinaho, J.; Latz, E.; Halle, A.; Petzold, G. C.; Town, T.; Morgan, D.; Shinohara, M. L.; Perry, V. H.; Holmes, C.; Bazan, N. G.; Brooks, D. J.; Hunot, S.; Joseph, B.; Deigendesch, N.; Garaschuk, O.; Boddeke, E.; Dinarello, C. A.; Breitner, J. C.; Cole, G. M.; Golenbock, D. T.; Kummer, M. P. Neuroinflammation in Alzheimer's Disease HHS Public Access. *Lancet Neurol.* **2015**, *14* (4), 388−405.
(20) Soto-Rojas, L. O.; Pacheco-Herrero, M.; Martínez-Gómez, P. A.; Campa-Córdoba, B. B.; Apátiga-Pérez, R.; Villegas-Rojas, M. M.; Harrington, C. R.; de la Cruz, F.; Garcés-Ramírez, L.; Luna-Muñoz, J. The Neurovascular Unit Dysfunction in Alzheimer's Disease. *Int. J. Mol. Sci.* **2021**, *22* (4), 2022.
(21) Lau, S.-F. F.; Cao, H.; Fu, A. K. Y. Y.; Ip, N. Y. Single-Nucleus Transcriptome Analysis Reveals Dysregulation of Angiogenic Endothelial Cells and Neuroprotective Glia in Alzheimer's Disease. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117* (41), 25800−25809.
(22) Ramanathan, A.; Nelson, A. R.; Sagare, A. P.; Zlokovic, B. V. Impaired Vascular-Mediated Clearance of Brain Amyloid Beta in Alzheimer's Disease: The Role, Regulation and Restoration of LRP1. *Front. Aging Neurosci.* **2015**, *7* (JUL), 1−12.
(23) Zhang, Y.; Sun, J.; Zhu, S.; Xu, T.; Lu, J.; Han, H.; Zhou, C.; Yan, J. The Role of Rhynchophylline in Alleviating Early Brain Injury Following Subarachnoid Hemorrhage in Rats. *Brain Res.* **2016**, *1631*, 92−100.
(24) Galatro, T. F.; Holtman, I. R.; Lerario, A. M.; Vainchtein, I. D.; Brouwer, N.; Sola, P. R.; Veras, M. M.; Pereira, T. F.; Leite, R. E. P.; Möller, T.; Wes, P. D.; Sogayar, M. C.; Laman, J. D.; Den Dunnen, W.; Pasqualucci, C. A.; Oba-Shinjo, S. M.; Boddeke, E. W. G. M.; Marie, S. K. N.; Eggen, B. J. L. Transcriptomic Analysis of Purified Human Cortical Microglia Reveals Age-Associated Changes. *Nat. Neurosci.* **2017**, *20* (8), 1162−1171.
(25) Mendes, M. S.; Majewska, A. K. An Overview of Microglia Ontogeny and Maturation in the Homeostatic and Pathological Brain. *Eur. J. Neurosci.* **2021**, *53*, 3525.
(26) Keren-Shaul, H.; Spinrad, A.; Weiner, A.; Matcovitch-Natan, O.; Dvir-Szternfeld, R.; Ulland, T. K.; David, E.; Baruch, K.; Lara-Astaiso, D.; Toth, B.; Itzkovitz, S.; Colonna, M.; Schwartz, M.; Amit, I. A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. *Cell* **2017**, *169* (7), 1276−1290.e17.
(27) Krasemann, S.; Madore, C.; Cialic, R.; Baufeld, C.; Calcagno, N.; El Fatimy, R.; Beckers, L.; O'Loughlin, E.; Xu, Y.; Fanek, Z.; Greco, D. J.; Smith, S. T.; Tweet, G.; Humulock, Z.; Zrzavy, T.; Conde-Sanroman, P.; Gacias, M.; Weng, Z.; Chen, H.; Tjon, E.; Mazaheri, F.; Hartmann, K.; Madi, A.; Ulrich, J. D.; Glatzel, M.; Worthmann, A.; Heeren, J.; Budnik, B.; Lemere, C.; Ikezu, T.; Heppner, F. L.; Litvak, V.; Holtzman, D. M.; Lassmann, H.; Weiner, H. L.; Ochando, J.; Haass, C.; Butovsky, O. The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. *Immunity* **2017**, *47* (3), 566−581.e9.
(28) Lau, S. F.; Chen, C.; Fu, W. Y.; Qu, J. Y.; Cheung, T. H.; Fu, A. K. Y.; Ip, N. Y. IL-33-PU.1 Transcriptome Reprogramming Drives Functional State Transition and Clearance Activity of Microglia in Alzheimer's Disease. *Cell Rep.* **2020**, *31* (3), 107530.
(29) Fu, A. K. Y.; Hung, K. W.; Yuen, M. Y. F.; Zhou, X.; Mak, D. S. Y.; Chan, I. C. W.; Cheung, T. H.; Zhang, B.; Fu, W. Y.; Liew, F. Y.; Ip, N. Y. IL-33 Ameliorates Alzheimer's Disease-like Pathology and Cognitive Decline. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113* (19), E2705−E2713.
(30) Xian, Y. F.; Su, Z. R.; Chen, J. N.; Lai, X. P.; Mao, Q. Q.; Cheng, C. H. K.; Ip, S. P.; Lin, Z. X. Isorhynchophylline Improves Learning and Memory Impairments Induced by D-Galactose in Mice. *Neurochem. Int.* **2014**, *76*, 42−49.
(31) Yang, Y.; Ji, W.-g.; Zhu, Z.-r.; Wu, Y.-l.; Zhang, Z.-y.; Qu, S.-c. Rhynchophylline Suppresses Soluble Aβ1−42-Induced Impairment of Spatial Cognition Function via Inhibiting Excessive Activation of Extrasynaptic NR2B-Containing NMDA Receptors. *Neuropharmacology* **2018**, *135*, 100−112.
(32) Kania, A.; Klein, R. Mechanisms of Ephrin-Eph Signalling in Development, Physiology and Disease. *Nat. Rev. Mol. Cell Biol.* **2016**, *17* (4), 240−256.
(33) Shen, L.; Kim, S.; Risacher, S. L.; Nho, K.; Swaminathan, S.; West, J. D.; Foroud, T.; Pankratz, N.; Moore, J. H.; Sloan, C. D.; Huentelman, M. J.; Craig, D. W.; DeChairo, B. M.; Potkin, S. G.; Jack, C. R.; Weiner, M. W.; Saykin, A. J. Whole Genome Association Study of Brain-Wide Imaging Phenotypes for Identifying Quantitative Trait Loci in MCI and AD: A Study of the ADNI Cohort. *NeuroImage* **2010**, *53* (3), 1051−1063.
(34) Rosenberger, A. F. N.; Rozemuller, A. J. M.; van der Flier, W. M.; Scheltens, P.; van der Vies, S. M.; Hoozemans, J. J. M. Altered Distribution of the EphA4 Kinase in Hippocampal Brain Tissue of Patients with Alzheimer's Disease Correlates with Pathology. *Acta Neuropathol. Commun.* **2014**, *2* (1), 1−13.
(35) Wei, H.-X.; Yao, P.-S.; Chen, P.-P.; Guan, J.-H.; Zhuang, J.-H.; Zhu, J.-B.; Wu, G.; Yang, J.-S. Neuronal EphA4 Regulates OGD/R-Induced Apoptosis by Promoting Alternative Activation of Microglia. *Inflammation* **2019**, *42* (2), 572−585.

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