



KBN2201 Reduces Amyloid Pathology And Neuroinflammation While Promoting Neurogenesis In Late-stage 5xFAD Mice

Mi Ran Choi^{a,b}, Sun-Young Lee^a, Jong Chul Kim^c, Moonhang Kim^a, Seok-Hwan Chang^a, Jong Sung Kim^a, Joon-Suk Park^c, and Sang-Rae Lee^{a,b,d,e,f}

*Correspondance: lsr21@ajou.ac.kr

^aEfficacy Test Center for Mental & Behavioral Disorders (MBD), Ajou University Hospital, Suwon, 16499, Republic of Korea; ^bLaboratory Animal Research Center, Ajou University School of Medicine, Suwon, 16499, Republic of Korea; ^cPreclinical Research Center, Daegu-Gyeongbuk Medical Innovation Foundation (K-MEDI hub), Daegu, 41061, Republic of Korea; ^dDepartment of Pharmacology, Ajou University School of Medicine, Suwon, 16499, Republic of Korea; ^eBK21 R&E Initiative for Advanced Precision Medicine, Suwon, 16499, Republic of Korea; ^fDepartment of Biomedical Sciences, Graduate School of Ajou University, Suwon, 16499, Republic of Korea

KBN2201 attenuates AD pathology and improves memory in late-stage 5xFAD mice

Introduction

- Alzheimer's disease (AD) is the most common form of dementia, characterized by progressive memory loss, cognitive decline, and behavioral changes. Hallmark neuropathologies include extracellular amyloid- β (A β) plaques, intracellular tau tangles, synaptic loss, and neuroinflammation. Because AD progression involves multiple interconnected pathological processes rather than a single molecular defect, multi-target therapeutic strategies may provide advantages over single-pathway approaches.
- KBN2201, a small-molecule derivative of 2-hydroxy-4-(trifluoromethyl)benzoic acid, has shown multimodal activity in vitro, including acetylcholinesterase inhibition, increased acetylcholine levels, suppression of microglial nitric oxide production, and downregulation of AD-related genes.
- To evaluate its in vivo therapeutic potential, we used aged 5xFAD mice, which exhibit robust amyloid deposition, gliosis, synaptic alterations, and cognitive impairment resembling advanced AD pathology.
- This study investigated whether orally administered KBN2201 can penetrate the brain and ameliorate AD-related pathological and cognitive deficits in a clinically relevant late-stage AD model.

Materials and Methods

- Animals:** Nine-month-old male transgenic 5xFAD mice received daily oral administration of KBN2201 (5 or 20 mg/kg) for 12 weeks. Age-matched vehicle-treated mice (Veh) were used as controls.
- Histology and immunostaining:** Brain sections were analyzed for A β plaques (Thioflavin S, 4G8), neuroinflammation (GFAP, Iba1), neuronal integrity (NeuN, MAP2), and neurogenesis-related markers (DCX, Ki67).
- Western blot:** Hippocampal lysates were analyzed for APP processing-related proteins and autophagy-lysosomal pathway markers. Protein expression levels were normalized to GAPDH.
- Behavioral testing:** Spatial working memory and recognition memory were evaluated using the Y-maze and novel object recognition (NOR) test.

Results

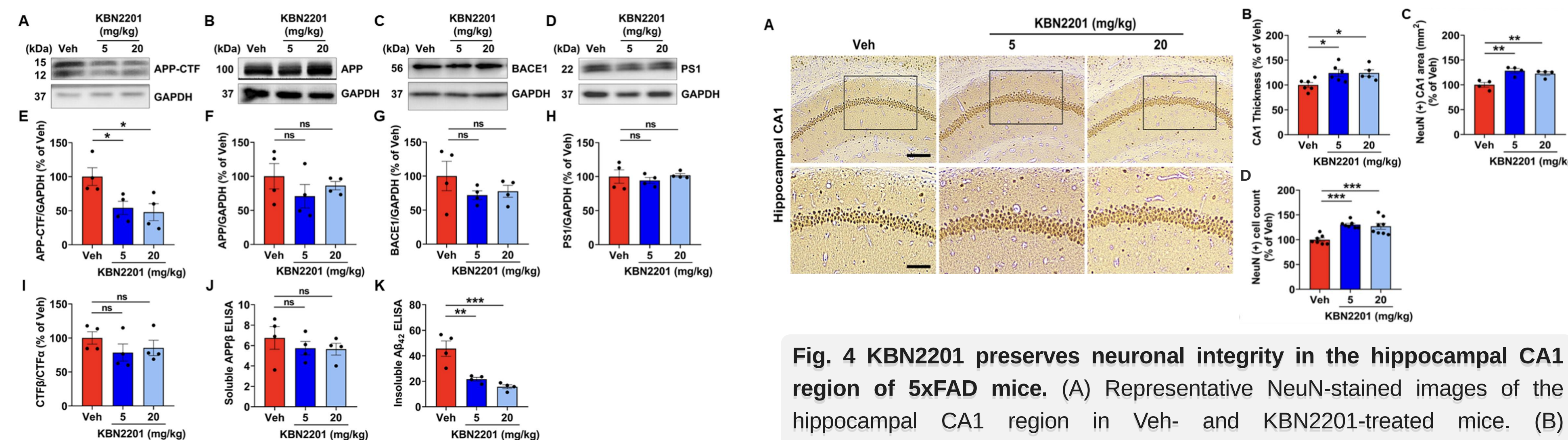


Fig. 4 KBN2201 preserves neuronal integrity in the hippocampal CA1 region of 5xFAD mice. (A) Representative NeuN-stained images of the hippocampal CA1 region in Veh- and KBN2201-treated mice. (B) Quantification of CA1 layer thickness. (C) Measurement of NeuN-positive area in the CA1 region. (D) Quantification of NeuN-positive cell counts in the CA1 region. Data are presented as mean $\pm$ SEM. * p < 0.05 and ** p < 0.01 vs. Veh.

Fig. 1 KBN2201 treatment reduces the levels of APP-CTF and insoluble A β 42 in the hippocampus of 5xFAD mice. (A-D) Representative Western blots of APP-CTFs (A), APP (B), BACE1 (C), and PS1 (D). (E-H) Quantification of APP-CTFs (E), APP (F), BACE1 (G), and PS1 (H), normalized to GAPDH. (I) Quantification of the CTF β /CTF α ratio. (J-K) Soluble sAPP β (J) and insoluble A β 42 (K) levels measured by ELISA. Data are presented as mean $\pm$ SEM. * p < 0.05, ** p < 0.01, and *** p < 0.001 vs. Veh.

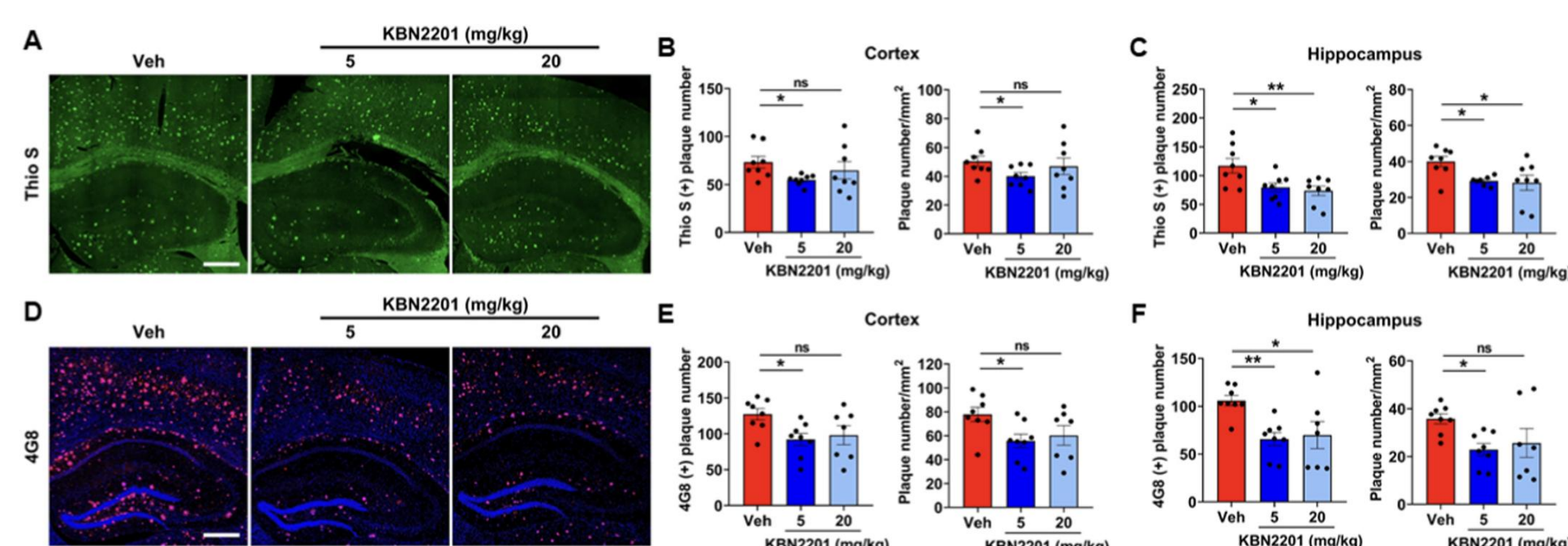


Fig. 2 KBN2201 reduces A β plaque burden in the cortex and hippocampus of 5xFAD mice. (A) Representative Thio S images showing fibrillar A β plaques in the cortex and hippocampus. (B-C) Quantification of Thioflavin S-positive plaque number and density in the cortex (B) and hippocampus (C). (D) Representative 4G8 immunofluorescence images showing total A β deposition. (E-F) Quantification of 4G8-positive plaque number and density in the cortex (E) and hippocampus (F). Data are presented as mean $\pm$ SEM. * p < 0.05 and ** p < 0.01 vs. Veh.

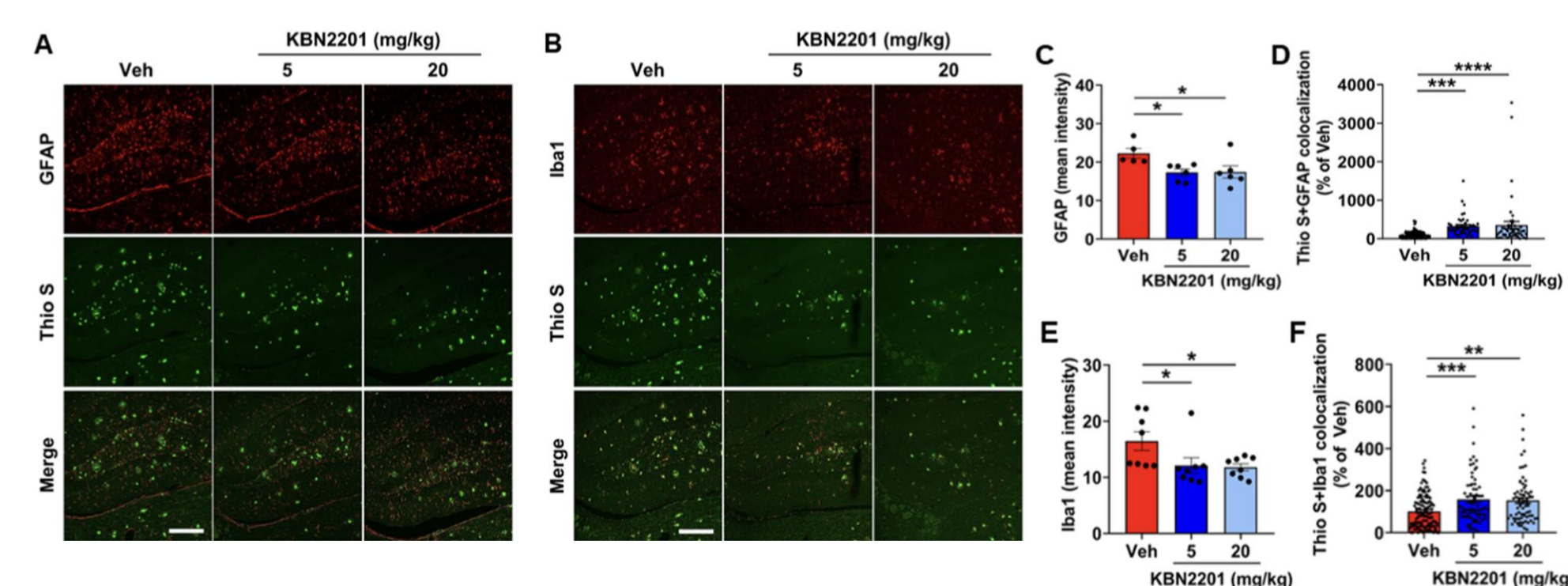


Fig. 3 KBN2201 reduces astrocytic and microglial activation in the hippocampus of 5xFAD mice. (A-B) Representative immunofluorescence images of GFAP-positive astrocytes (A) and Iba1-positive microglia (B) with Thio S-labeled A β plaques in the hippocampus. (C) Quantification of GFAP fluorescence intensity. (D) Quantification of GFAP and Thio S co-localization. (E) Quantification of Iba1 fluorescence intensity. (F) Quantification of Iba1 and Thio S co-localization. Data are presented as mean $\pm$ SEM. * p < 0.05 and ** p < 0.01 vs. Veh.

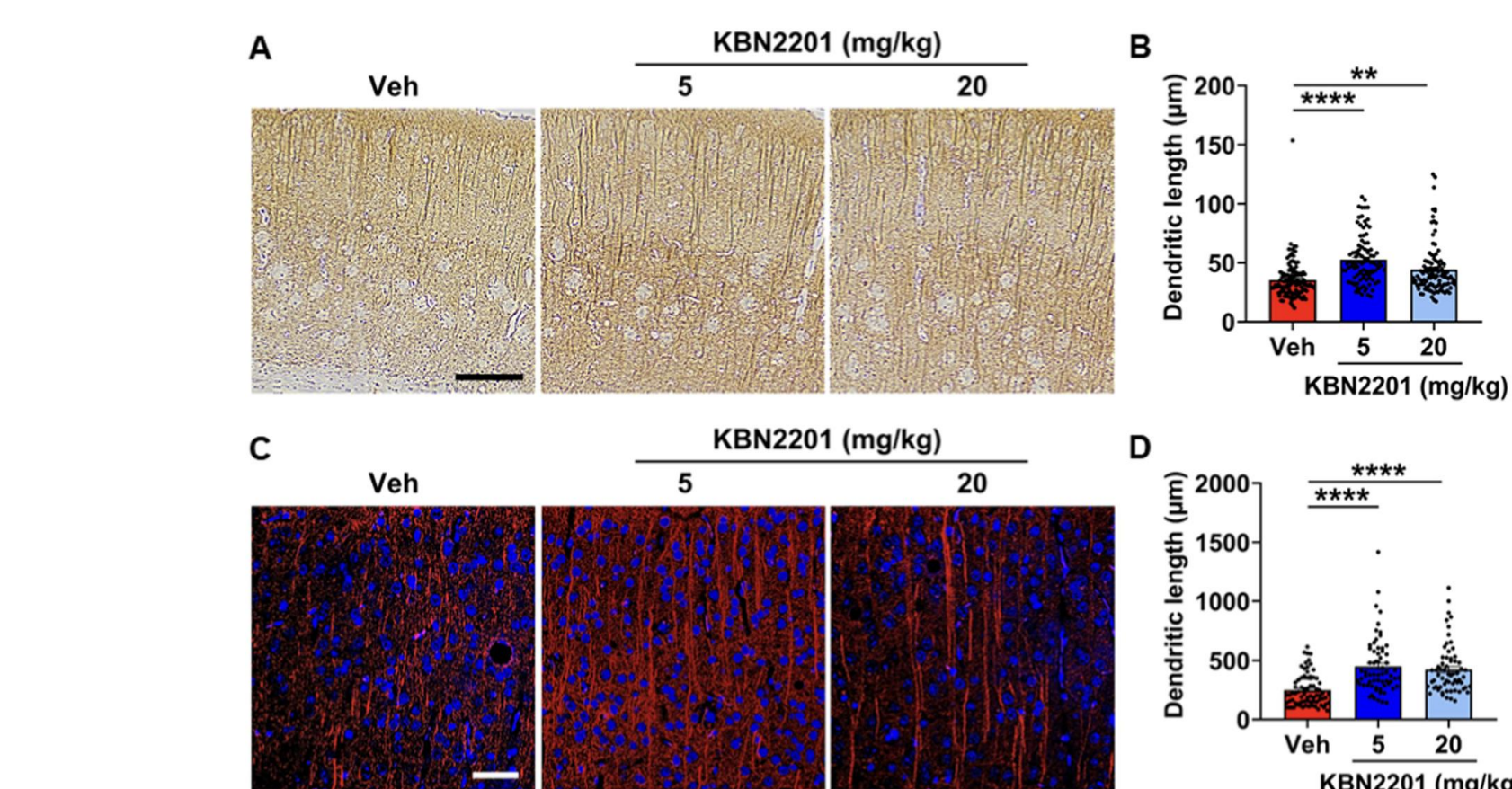


Fig. 5 KBN2201 restores dendritic length in the cortex of 5xFAD mice. (A) Representative MAP2 DAB-stained cortical sections showing dendritic morphology. (B) Quantification of cortical dendritic length based on MAP2 DAB staining. (C) Representative MAP2 immunofluorescence images of the cortex. (D) Quantification of dendritic length from MAP2 immunofluorescence images. Data are presented as mean $\pm$ SEM. ** p < 0.01 and **** p < 0.0001 vs. Veh.

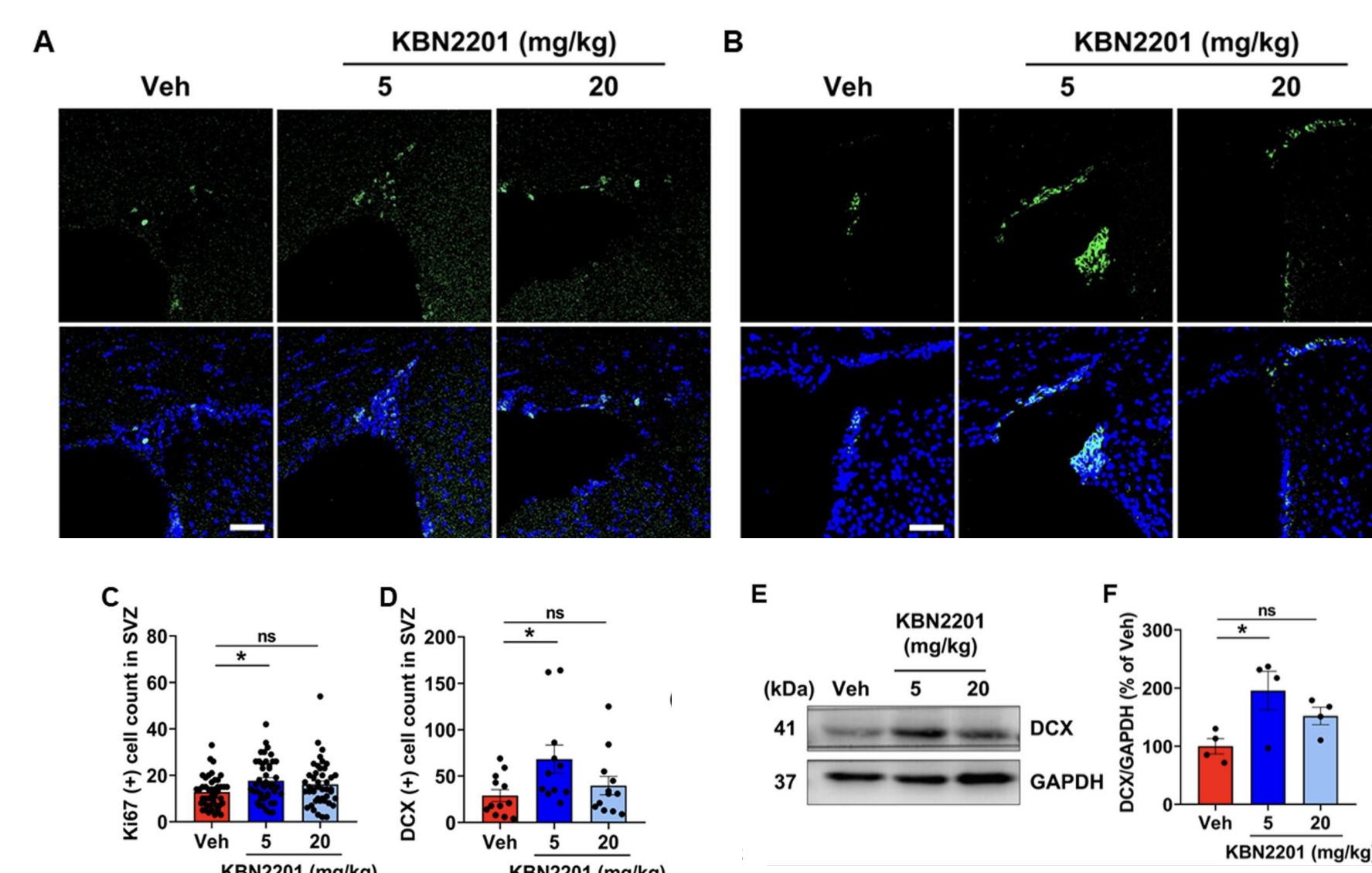


Fig. 6 KBN2201 is associated with increased neurogenesis-related markers in neurogenic regions of 5xFAD mice. (A-B) Representative immunofluorescence images of Ki67-positive proliferating cells (A) and DCX-positive immature neurons (B) in the SVZ. (C-D) Quantification of Ki67-positive (C) and DCX-positive (D) cell numbers in the SVZ. (E) Representative Western blot images showing DCX expression in hippocampal lysates. (F) Quantification of DCX levels normalized to GAPDH. Data are presented as mean $\pm$ SEM. * p < 0.05 vs. Veh.

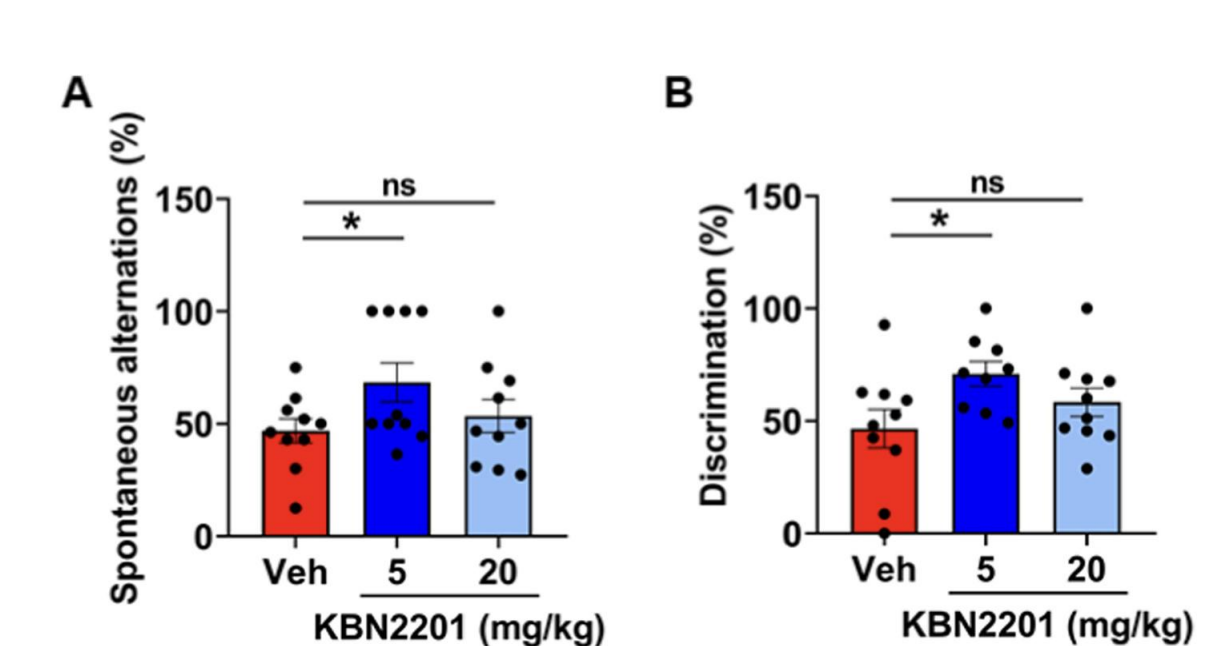


Fig. 7 KBN2201 improves performance in behavioral tests in 5xFAD mice. (A) Performance in the Y-maze spontaneous alternation test. Spontaneous alternation percentage in Veh- and KBN2201-treated mice. (B) Performance in the NOR test. Discrimination index measured during the initial 5-min test phase. Data are presented as mean $\pm$ SEM. * p < 0.05 vs. Veh.

Conclusions

- KBN2201 showed therapeutic efficacy in aged 5xFAD mice by reducing A β pathology and A β -associated neuroinflammation.
- Oral treatment preserved neuronal and dendritic integrity and increased neurogenesis-related markers in the SVZ and hippocampus.
- KBN2201 improved spatial working and recognition memory, with greater behavioral improvement observed in the 5 mg/kg group.
- These findings support KBN2201 as a promising multi-target therapeutic candidate for late-stage Alzheimer's disease.

Reference

- Lee, SY, Kim, JC, Song, J, *et al.*, KBN2201 attenuates A β pathology and neuroinflammation while promoting neuroprotective and neurogenesis-related changes in a late-stage 5xFAD mouse model of Alzheimer's disease. *Neuropharmacology*. 2026;294:110970.

Acknowledgements

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