



The combined effects of NK4146, a live biotherapeutic, with Lecanemab or Donepezil on Alzheimer's disease-related symptoms in 5xFAD-transgenic mice

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ABSTRACT

Gut microbiota is closely involved with the pathogenesis of Alzheimer's disease (AD). Oral administration of NK4146, a live biotherapeutic composed of *Limosilactobacillus mucosae* NK41 and *Bifidobacterium longum* NK46, alleviated cognitive impairment-like behaviors in 5xFAD-transgenic mice, accompanied by reduction in A β accumulation, amyloid precursor protein, β -secretase, γ -secretase, caspase-3, and TNF- α expression in both the brain and intestine and . NK4146 treatment also significantly improved gut dysbiosis. Notably, fecal microbiota transplantation (FMT) from NK4146-treated 5xFAD-transgenic mice to antibiotics-treated mice did not induce cognitive impairment-related symptoms, while FMT from saline-treated 5xFAD-transgenic mice caused cognitive impairment.

Lecanemab treatment alone alleviated cognitive impairment-like behaviors and reduced A β accumulation, but not A β -biosynthesizing enzymes, in 5xFAD-transgenic mice. Treatment with Donepezil alone alleviated cognitive impairment-like behaviors in 5xFAD-transgenic mice, while the A β accumulation was not reduced. Combined treatment of NK4146 and Lecanemab (NL) synergistically reduced cognitive impairment-related behaviors, A β accumulation, and the expression of amyloid precursor protein (APP), A β -biosynthesizing enzymes, and proinflammatory cytokines in 5xFAD-transgenic mice. Orally administered NK4146 or NL reduced the total abundance of gram-negative Verrucomicrobiota, Cyanobacteria, and Pseudomonadota populations in 5xFAD-transgenic mice.

Combined treatment of NK4146 and donepezil additively reduced cognitive impairment-related behaviors, donepezil did not increase NK4146-dependent suppression of A β accumulation and A β -biosynthesizing enzyme expression.

METHODS

1) Test agents:

NK4146, **Lecanemab (Lm)**, **donepezil (DP)**, **NL** (NK4146+Lm), and **NP**

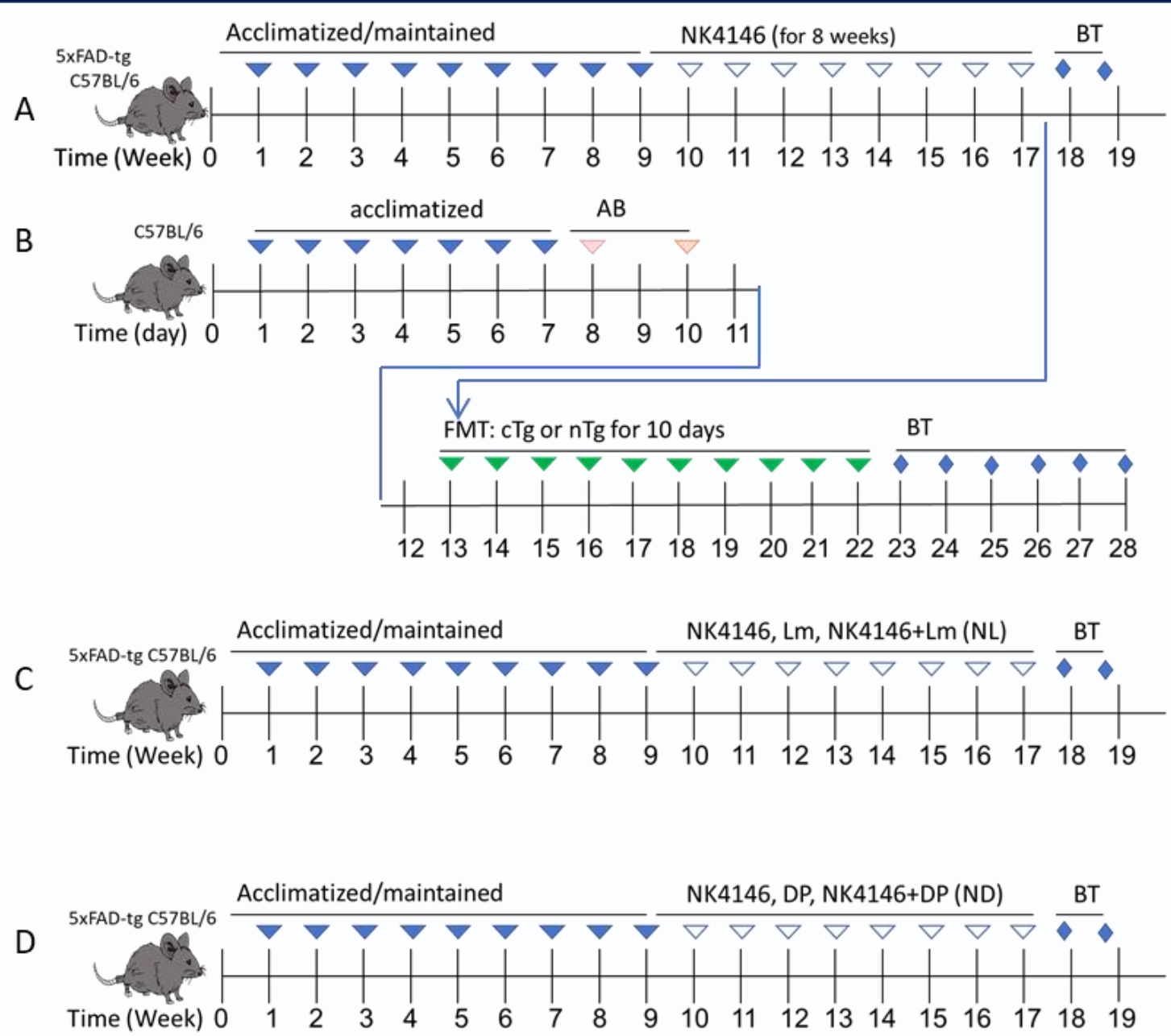
2) 5xFAD-transgenic mice (male, 2 months-old, Jackson Lab)

3) Animal experiments
A: NK4146 (8 weeks-treated)

B: fecal microbiota of A

C: NK4146, Lm, and NL

D: NK4146, DP, and NP



CONCLUSION

- NK4146 may alleviate AD-related pathology through the suppression of gut microbiota-involved A β synthesizing process in both the intestine and brain though the microbiota-gut-brain axis.**
- Lecanemab treatment alone alleviated cognitive impairment-like behaviors and reduced A β accumulation, but not A β -biosynthesizing enzymes, in 5xFAD-transgenic mice.
- Treatment with donepezil alone alleviated cognitive impairment-like behaviors in 5xFAD-transgenic mice, while the A β accumulation was not reduced.
- The combination of NK4146 with Lecanemab synergistically alleviated AD through gut microbiota-involved suppression of A β biosynthesis and neuroinflammation by NK4146 and A β expression and accumulation by Lecanemab, or inflammation by donepezil.**
- The combination of NK4146 with donepezil additively alleviated AD through gut microbiota-involved suppression of A β biosynthesis and neuroinflammation by NK4146 and of inflammation by donepezil.**

BACKGROUND

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by amyloid- β (A β) accumulation, neuroinflammation, synaptic dysfunction, and cognitive decline. Neuropathological hallmarks of AD include the accumulation of insoluble amyloid- β (A β) plaques and hyperphosphorylated tau neurofibrillary tangles, which elicit neuroinflammatory responses and disrupt cholinergic neurotransmission. Recent studies suggest that gut microbiome is strongly linked to AD. Current therapeutic strategies for AD include acetylcholinesterase inhibitors (such as donepezil), cholinergic agonists (such as galantamine), anti-inflammatory agents (such as aspirin), and anti-A β monoclonal antibodies (such as Lecanemab). *Limosilactobacillus* (*Lactobacillus*) *mucosae* NK41, *Bifidobacterium longum* NK46, and their (4:1) mix (NK4146) attenuate cognitive impairment, gut inflammation, and gut dysbiosis in LPS-impaired, 5xFAD-transgenic, and aged mice (H. J. Lee et al., 2019; Ma et al., 2023). However, whether the beneficial effects of NK4146 on microbiota-associated AD-related pathology are linked to gut-brain signaling remains unclear. Therefore, we investigated the effects of NK4146, alone or in combination with Lecanemab or donepezil, on AD-related pathology, microbiota modulation, and amyloid- β accumulation in 5xFAD-transgenic mice.

RESULTS 1) Effects of NK4146

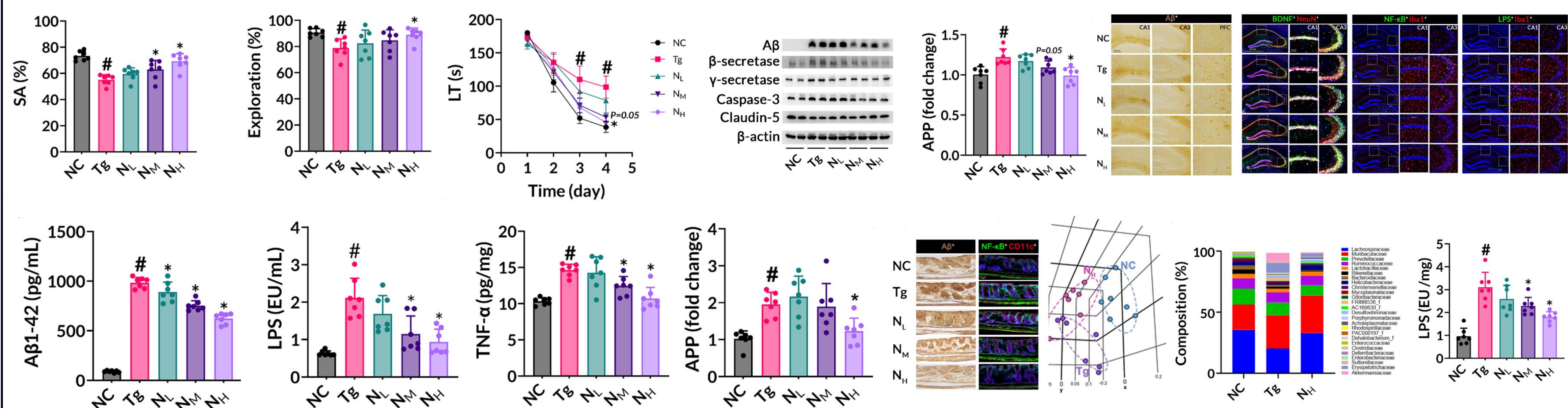


Figure 1. Effects of NK4146 on AD-related symptoms in 5xFAD mice. Test agents (Tg, saline; N_L, 0.5×10⁹ CFU/mice/day of NK4146; N_M, 2×10⁹ CFU/mice/day of NK4146; N_H, 1×10⁹ CFU/mice/day of NK4146) were orally administered in 5xFAD-transgenic mice for 8 weeks (6 days/week). Normal control mice (NC) were treated with saline in C57BL/6 mice instead of test agents. Data values were described as mean ± SD (n = 7). #p<0.05 vs. NC. *P<0.05 vs. Tg. Spontaneous alternation (SA) in the Y-maze test, exploration in the novel object recognition test, latency time (LT) in the Barnes maze test.

RESULTS 2) Effects of NK4146 + Lecanemab

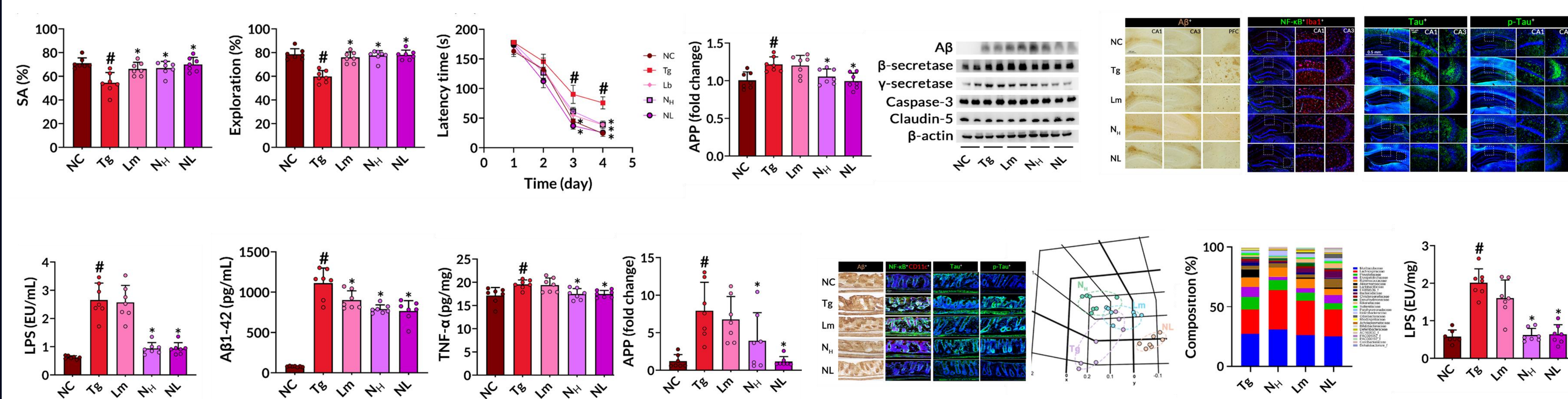


Figure 2. Effects of NK4146, Lecanemab, and their mix (NL) on AD-related symptoms in 5xFAD mice. Test agents (Tg, saline; Lm, 3 mg/kg/week of Lecanemab; N_L, 1×10⁹ CFU/mice/day of NK4146; NL, mix of 3 mg/kg/week of Lecanemab and 1×10⁹ CFU/mice/day of NK4146) were orally administered in 5xFAD-transgenic mice for 8 weeks (6 days/week). Normal control mice (NC) were treated with saline in C57BL/6 mice instead of test agents. Data values were described as mean ± SD (n = 7). #p<0.05 vs. NC. *P<0.05 vs. Tg.

RESULTS 3) Effects of NK4146 + donepezil

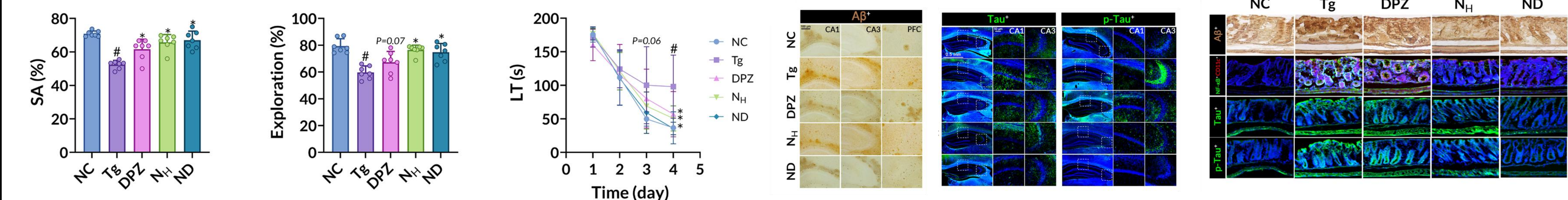


Figure 3. Effects of NK4146, donepezil (DPZ), and mixture (ND) on AD-related symptoms in 5xFAD mice. Test agents (Tg, saline; DPZ, 2.1 mg/kg/day of donepezil; N_H, 1×10⁹ CFU/mice/day of NK4146; ND, mix of 2.1 mg/kg/day of donepezil and 1×10⁹ CFU/mice/day of NK4146) were orally administered in 5xFAD-transgenic mice for 8 weeks (6 days/week). Normal control mice (NC) were treated with saline in C57BL/6 mice instead of test agents. Data values were described as mean ± SD (n = 7). #p<0.05 vs. NC. *P<0.05 vs. Tg.

REFERENCES

- Lee, H. J., Lee, K. E., Kim, J. K., & Kim, D. H. (2019). Suppression of gut dysbiosis by Bifidobacterium longum alleviates cognitive decline in 5xFAD transgenic and aged mice. *Sci Rep*, 9(1), 11814.
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