Small molecule of adiponectin receptor agonist—AdipoRon—for Alzheimer disease: abridged secondary publication

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KEY MESSAGES

1. Chronic oral administration of AdipoRon reverses spatial learning and memory performance in an Alzheimer mouse model.
2. AdipoRon crosses the blood-brain barrier and reaches maximum concentration 2 hours after oral administration.
3. AdipoRon enhances neuronal insulin sensitivity and reduces amyloid-β deposition.
4. AdipoRon suppresses amyloid-β-mediated neuroinflammatory responses, exerting protective effects to neurons and synapses in Alzheimer mouse model.

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Introduction

Alzheimer disease (AD) is the most common neurodegenerative cause leading to dementia. The characteristic neuropathological features of AD include amyloid-β-forming neuritic plaques, hyperphosphorylated tau-forming intracellular neurofibrillary tangles, neuroinflammation, and neuronal loss in the hippocampus and frontal cortex. In AD drug invention, immunotherapy-based targeting amyloid-β and tau treatment have demonstrated promising results in mouse models. However, these attempts failed to improve cognitive outcomes in clinical trials. Recently, insulin sensitising and anti-diabetic approaches have provided a new therapeutic strategy to treat AD.

Insulin resistance is a common pathogenesis of both type 2 diabetes mellitus and AD. Diabetic mouse models demonstrated Alzheimer-like alterations and provided evidence that brain insulin resistance is likely to be the main cause of the AD-like pathogenesis.[1] Postmortem AD brains and brains from AD mouse models also showed increased cerebral insulin resistance. Brain insulin resistance increases Aβ accumulation and tau hyperphosphorylation. Therefore, strategy enhancing insulin sensitivity becomes a promising therapeutic method to treat AD.

Adiponectin is an insulin-sensitising adipokine with anti-inflammatory and anti-oxidative effects. Hypoadiponectinaemia is a risk factor of insulin resistance-associated type 2 diabetes mellitus. Findings are inconclusive in whether high or low level of adiponectin is associated with AD. We have revealed that chronic adiponectin deficiency results in AD-like pathologies and cognitive impairments in aged mice, and these mice develop cerebral insulin resistance with reduced hippocampal insulin sensitivity.[2] AdipoRon is a newly invented adiponectin receptor agonist that can be orally administered. It can improve glucose uptake, lipid metabolism, and insulin sensitivity in mammalian cells and in mice. Life span has been increased in high-fat-diet-fed *db/db* mice (an animal model for type 2 diabetes mellitus and obesity) through metabolic improvement after oral administrating AdipoRon. Therefore, we hypothesise that AdipoRon can be a promising therapeutic drug for AD by enhancing insulin sensitivity and suppressing neuroinflammation. In this project, we demonstrated that oral administration of AdipoRon reversed cognitive deficits in transgenic AD mice. AdipoRon-treated AD mice showed increased neuronal insulin sensitisation, ameliorated neuropathologies, and reduced neuroinflammatory responses.

Methods

To determine if AdipoRon is a blood-brain barrier penetrant, pharmacokinetics of AdipoRon in mice plasma and the brain were determined quantitatively. C57BL6/N mice (20-25 g) were randomly divided into seven groups with three mice in each group. Each mouse was orally administered 50 mg/kg AdipoRon dissolved in corn oil. The mice were euthanised at 0, 0.5, 1, 2, 4, 8, or 12 hours after drug administration, and blood and brain tissue were collected. Plasma samples were obtained by centrifuging the blood at 3500 × g for 5 minutes, and brain tissue was homogenised and processed to extract the content.

Finally, 10 μL of the reconstituted solution was analysed by LC-MS/MS. The concentrations of AdipoRon in different samples were determined by coupled to an API3200 triple quadrupole MS (Sciex, Ontario, Canada) that was equipped with a TurboIonSpray ion source.

Five-month-old 5xFAD mice were fed with AdipoRon (50 mg/kg bodyweight) by oral gavage daily. Littermate controls were fed with vehicle. After 3 months of administration, mice were subjected to behavioural analysis including Morris water maze test for their spatial learning and memory functions, open field test for anxiety level, and fear-conditioning test for hippocampal-dependent memory of aversive stimuli. >10 animals underwent these behavioural tests.

To determine whether AdipoRon treatment enhances insulin signalling activities, the hippocampus was dissected and was snap-frozen for western blot analysis of insulin signalling molecules. Hippocampal insulin sensitivity was also examined by injecting recombinant insulin to the right hippocampus with or without AdipoRon administration using stereotaxic instruments. Induction of insulin signalling in the hippocampus was determined by western blot analysis.

Neuropathological changes, in the control and AdipoRon-treated AD mice, including amyloid-β deposition, microgliosis, astrogliosis, and neuronal loss were examined by immunofluorescent staining using corresponding primary antibodies. Golgi-Cox staining was performed to visualise the spine density of the hippocampal apical dendrites.

Statistical analyses were performed with GraphPad Prism 6 (GraphPad Software). For the Morris water maze test, dataset was analysed by two-way ANOVA. Other behavioural tests were analysed by one-way ANOVA. In other experiments, between-group differences were determined with one-way ANOVA, followed by Bonferroni post hoc test. Alternatively, the mean significant difference between two groups was determined with two-tailed unpaired Student's *t*-test. Statistical significance was defined as $P<0.05$.

Results

AdipoRon crosses the blood-brain barrier and increases AMPK phosphorylation

The pharmacokinetics of AdipoRon in the plasma and the brain were studied by LC-MS/MS analysis. Plasma AdipoRon reached the maximum level 2 hours after oral gavage. AdipoRon was detected in the brain samples, with the highest level at 2 hours after oral gavage. This provides concrete evidence that AdipoRon can cross the blood-brain barrier. We then investigated if AdipoRon activated adiponectin signalling by increasing AMPK phosphorylation in the hippocampal lysates. Western blot analysis indicated that the pAMPK levels of the hippocampus were increased 2 hours after AdipoRon treatment compared to the vehicle treatment.

AdipoRon reverses memory and cognitive functions

To investigate if chronic AdipoRon administration can improve cognitive and memory functions of AD mouse model, 5xFAD mice at age 5.5 months were fed with AdipoRon daily by oral gavage for 3 months. The mice were then examined with the anxiety levels by open field test, in which less time spent at the centre indicates higher anxiety level for the mice. Interestingly, AdipoRon-treated mice spent significantly increased time than vehicle-treated mice. This result showed that AdipoRon reduced anxiety levels in the AD mouse model.

To assess the learning and memory functions, the Morris water maze test was performed. Analysis confirmed an overall learning and memory impairment in 9-month-old 5xFAD mice compared to wildtype mice. 5xFAD with chronic AdipoRon treatment reversed the overall learning and memory performance with shorter escape latency after 5 days of the hidden test. Furthermore, the probe test revealed AdipoRon-treated 5xFAD mice spent more time in the target quadrant compared with the vehicle-treated littermates.

AdipoRon treatment significantly improved the performance of 5xFAD mice in the contextual fear conditioning tests compared with the vehicle-treated littermates. However, we did not observe improvement in the freezing response in the cued fear conditioning tasks. All these results indicate that AdipoRon improved hippocampus-dependent learning and memory functions in the AD mouse model.

AdipoRon enhances neuronal and cerebral insulin sensitisation

AdipoRon markedly increased the levels of Akt phosphorylation at serine 473 residue and GSK3β at serine 9 residue, comparable to the levels of wildtype mice. On the contrary, the phosphorylation of IRS-1 at serine 616, which related to insulin resistance, was reduced in the AdipoRon-treated 5xFAD mice. In the stereotaxic injection experiment, vehicle-treated 5xFAD showed insignificant increased of $pAkt^{S473}$ levels, whereas AdipoRon-treated 5xFAD mice showed a significant increase in the level of $pAkt^{S473}$. These data show that aberrant reduction of insulin signalling in 5xFAD mice was enhanced after oral administration of AdipoRon.

AdipoRon reduces amyloid pathology and neuroinflammation

To examine the effect of AdipoRon on amyloid-β deposition in the brain, we observed the levels

of amyloid-β plaques in mice subjected to the behavioural studies. Brain sections were stained with thioflavin S (ThioS) to visualise insoluble β-sheet amyloid-β deposits. Total number and amyloid-β loading found in the cerebral cortex and hippocampus were quantified. The amyloid-β loading and number of deposits was dramatically reduced in these brain regions of AdipoRon-administered 5xFAD mice compared with vehicle controls.

To investigate if AdipoRon reduced microgliosis and astrogliosis, the brain sections were immunostained with Iba1 (microglia marker) and GFAP (astrocyte marker). Our results indicated that both Iba1 and GFAP levels were reduced in the cerebral cortex and hippocampus of AdipoRon-treated 5xFAD mice compared with that of vehicle-treated 5xFAD mice. We also studied the levels of proinflammatory cytokines (IL1β and TNFα) by ELISA analysis. These cytokines have demonstrated detrimental effects on neurons in AD brains. The levels of IL1β and TNFα increased in the brains of 5xFAD mice compared with wildtype mice. Importantly, 5xFAD with chronic AdipoRon administration had reduction of IL1β and TNFα levels. These results demonstrated that AdipoRon reduced neuroinflammatory responses in the transgenic AD model.

AdipoRon restores neuronal loss and dendritic spine reduction

To quantify hippocampal neurons, we performed NeuN immunostaining to visualise CA1 neurons. We found an insignificant decrease of CA1 neurons in the vehicle-treated 5xFAD mice compared to wildtype, whereas AdipoRon-treated mice showed a comparable number of NeuN-stained neurons. Moreover, we examined if the memory improvement after AdipoRon administration associated with neuronal and synaptic changes. We performed double immunofluorescent staining of Ctip2 and Brn2 to label and quantified the layer V neurons in different mice group. 5xFAD mice had reduced layer V cortical neurons compared with wildtype mice. AdipoRon-treated 5xFAD mice had a significant increase of the layer V neurons compared with 5xFAD mice.

In the Golgi-Cox staining, we found an overall decrease in spine density in vehicle-treated 5xFAD mice relative to littermate wildtype. Importantly, AdipoRon-treated 5xFAD mice revealed a complete restoration of the spine deficit in apical dendrites of the CA1 layer.

Discussion

Orally administered adiponectin receptor agonist could cross the blood-brain barrier. Pharmacokinetic study demonstrated that AdipoRon reached the highest concentration in the brain and plasma simultaneously. However, the half-life of AdipoRon was short. The molecule was almost undetectable by LC-MS/MS 6 hours after oral administration. Therefore, the mice were fed with AdipoRon for 3 months. We provided concrete evidence supporting AdipoRon as a promising medication to treat AD. Long-term AdipoRon administration reduced neuropathologies in AD mice with significant improvement of cognitive and memory functions. However, structure modification of the molecule may be necessary to prolong the half-life of AdipoRon in order to maximise the therapeutic effects.

Apart from insulin sensitising, adiponectin is an anti-inflammatory agent, our results also demonstrated that AdipoRon could suppress neuroinflammatory responses by reducing activation of microglia and astrocyte. Activation of these glial cells is detrimental because the activated glia produces high levels of proinflammatory cytokines. These cytokines are toxic to neuron and can affect neuronal function.[3] From our data, cerebral proinflammatory cytokine levels were significantly reduced after AdipoRon administration. This provided an explanation that 5xFAD mice were protected from neuronal and synaptic reduction after treating with AdipoRon.

Reports indicated that adiponectin could promote hippocampal neurogenesis and dendritic spine formation.[4,5] From our data, AdipoRon prevented neuronal loss and spine reduction. However, it may also increase neurogenesis to increase the number of functional neurons and promotes spine formation. These require further study to investigate if AdipoRon can restore the number of neurons at the late stage of AD.

Conclusion

AdipoRon can reverse memory impairments, reduce anxiety levels, and improve neuropathology in AD mice. AdipoRon enhances neuronal insulin sensitivity and ameliorates insulin resistance in the hippocampus. AdipoRon can also reduce inflammatory responses and cytokines levels. These support the potential therapeutic effects of AdipoRon to treat AD.

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Disclosure

The results of this research have been previously published in:

1. Ng RC, Jian M, Ma OK, et al. Chronic oral administration of adipoRon reverses cognitive impairments and ameliorates neuropathology in an Alzheimer's disease mouse model. Mol Psychiatry 2020;10.1038/s41380-020-0701-0.

References

1. de la Monte SM. Brain insulin resistance and deficiency as therapeutic targets in Alzheimer's disease. Curr Alzheimer Res 2012;9:35-66.
2. Ng RC, Cheng OY, Jian M, et al. Chronic adiponectin deficiency leads to Alzheimer's disease-like cognitive impairments and pathologies through AMPK inactivation and cerebral insulin resistance in aged mice. Mol Neurodegener 2016;11:71.
3. Floden AM, Combs CK. Beta-amyloid stimulates murine postnatal and adult microglia cultures in a unique manner. J Neurosci 2006;26:4644-8.
4. Zhang D, Wang X, Lu XY. Adiponectin exerts neurotrophic effects on dendritic arborization, spinogenesis, and neurogenesis of the dentate gyrus of male mice. Endocrinology 2016;157:2853-69.
5. Song J, Kang SM, Kim E, Kim CH, Song HT, Lee JE. Adiponectin receptor-mediated signaling ameliorates cerebral cell damage and regulates the neurogenesis of neural stem cells at high glucose concentrations: an in vivo and in vitro study. Cell Death Dis 2015;6:e1844.