新乡医学院

科技活动月 研究成果展

A new metal ion chelator attenuates human Tau accumulation-induced neurodegeneration and memory deficits in mice

Background

Neuronal neurofibrillary tangles containing hyperphosphorylation Tau proteins are the unique hallmark of Alzheimer's Disease (AD). Metal ions deposition occurs with tangles in AD brain autopsy. Reduced metal ions can improve the pathology of AD. To explore whether abnormal phosphorylated Tau causes metal ion deposition, we overexpressed human full-length Tau (hTau) in hippocampal CA3 area of mice and primary cultured hippocampal neurons (CPHN) and found Tau accumulation induced iron deposition and activated calcineurin (CaN) that dephosphorylates glycogen synthase kinase 3 beta (GSK3β) mediating hyperphosphorylation Tau. Simultaneously activation of CaN dephosphorylates cyclic-AMP response binding protein (CREB) leads to synaptic deficits and memory impairment as shown in our previous study. This seems like a vicious circle exacerbating tauopathy. In the current study, we developed a new metal ion chelator that displayed a significant inhibitory effect on Tau phosphorylation and memory impairment via chelating iron ions in vivo and in vitro.

Authors

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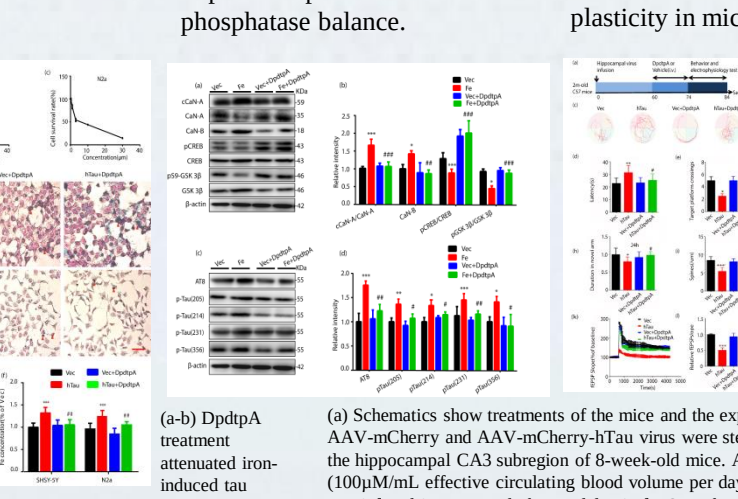
Methods

1.Cell Proliferation and Cytotoxicity Assays
2.Animals, stereotactic brain surgery and administration of drugs
3.Western blotting
4.Atomic absorption spectroscopy
5.Behavioral Test
7.Electrophysiological recording
8. Hippocampal primary neuron culture
9.Golgi staining
10.Perls Stain

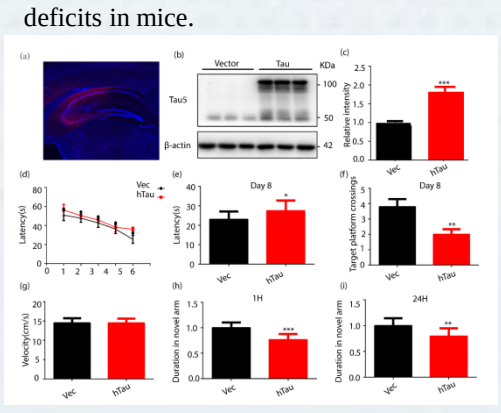
Results



Human tau (hTau) accumulation activated calcineurin (CaN), which could inactivate CREB leading synapse dysfunction and memory deficit; CaN activation could also dephosphorylate/activate GSK3β causing tau hyperphosphorylation and iron accumulation; The iron deposition could conversely activate CaN, and thus formed a vicious cycle between tau and iron deposition. Importantly, a new iron chelator could efficiently chelate iron and thus alleviate tau hyperphosphorylation/accumulation and memory loss.

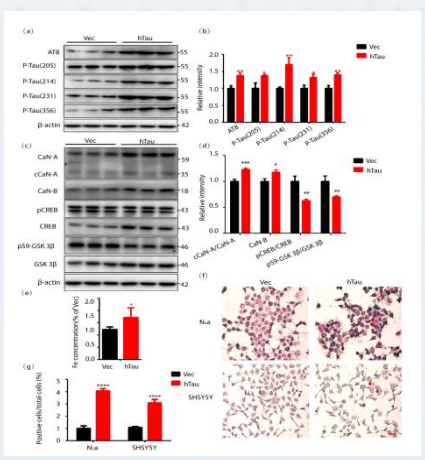
Results

Figure 1. Overexpressing hTau in hippocampal CA3 induces cognitive deficits in mice.



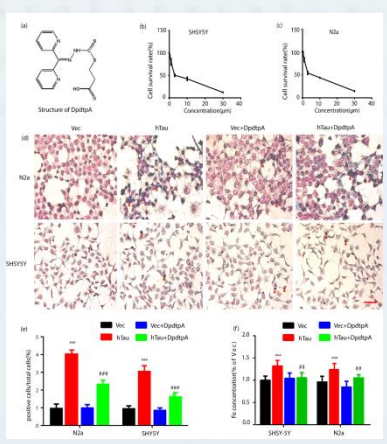
(a) AAV-mCherry-hTau virus was infused stereotaxically into the bilateral hippocampal CA3 regions of mice, and after 10 weeks, the expression of the virus vector in the hippocampal CA3 was identified by immunofluorescence imaging (scale bar 100 μm).
(b-c) The hippocampal CA3 subset was dissected and robust expression of the exogenous hTau protein (at ~100 kDa) with an increased constitutive mouse tau (at ~50 kDa) was confirmed by Western blotting.
(d) Overexpressing hTau in CA3 did not significantly affect the spatial learning of mice evidenced by the unchanged latency to find the hidden platform during 5 consecutive days Morris water maze (MWM) training.
(e-g) Overexpressing hTau in CA3 induced spatial memory deficit evidenced by the reduced latency to reach the platform quadrant (e) and the decreased crossings (f) in the target region during MWM probe test performed at day 7. Expressing hTau did not affect the motor function of the mice evidenced by the unchanged moving speed (g).
(h-i) Overexpressing hTau in CA3 decreased the duration time of the mice in the novel aim during Y-maze test performed at 1 h (h) and 24 h (i), respectively, after the training.

Figure 2. Overexpressing hTau increases iron level with tau accumulation and protein kinase and phosphatase imbalance both *in vitro* and *in vivo*.



(a-b) Overexpressing hTau in the hippocampal CA3 for 10 weeks not only increased total tau probed by Tau5 and hyperphosphorylated tau at multiple AD-associated sites.
(c-d) Overexpressing hTau in the hippocampal CA3 for 10 weeks induced imbalanced expression or posttranslational modifications of calcineurin (CaN) and GSK3β detected by Western blotting.
(e) Overexpressing hTau in the hippocampal CA3 for 10 weeks increased iron level in mice detected by atomic absorption spectrometry.
(f-g) Overexpressing hTau in N_2a and SHSY5Y cells for 48h increased iron level measured by Perls staining (scale bar 100 μm). The iron granules appeared as bright blue or blue-green aggregates, and the ratio of positive cells/total cells was presented.

Figure 3. DpdtpA alleviates abnormal iron accumulation induced by overexpressing hTau.

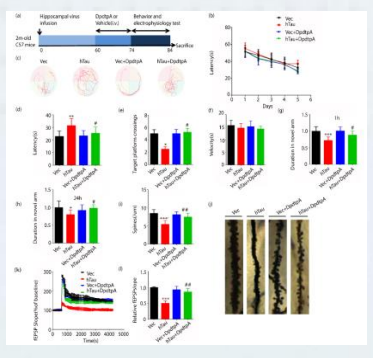


(a) Molecular formula of DpdtpA.
(b) The effect of DpdtpA on the proliferation of SHSY5Y cells.
(c) The effect of DpdtpA on the proliferation of N_2a cells.
(d) Treatment of N_2a-hTau and SHSY5Y-hTau cells with DpdtpA (scale bar 100 μm) for 24 h attenuated hTau-induced iron increase measured by atomic absorption spectrometry.
(e-f) Treatment of N_2a-hTau and SHSY5Y-hTau cells with DpdtpA for 24 h attenuated hTau-induced iron accumulation measured by by Perls staining (scale bar 100 μm). The percentage of positive cells with iron granules was analyzed.

Figure 4. DpdtpA attenuates excessive iron-induced tau hyperphosphorylation with improved protein kinase and phosphatase balance.

(a-b) DpdtpA treatment attenuated iron-induced tau hyperphosphorylation at multiple AD-associated sites, including AT8 epitope, Thr205, Ser214, Thr231, and Ser356.
(c-d) DpdtpA treatment attenuated iron-induced imbalance of CaN and GSK3β.

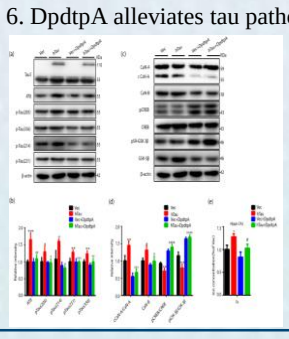
Figure 5. DpdtpA attenuates hTau-induced cognitive deficits with improved CREB activity and synaptic plasticity in mice



(a) Schematics show treatments of the mice and the experimental procedure. AAV-mCherry and AAV-mCherry-hTau virus were stereotaxically infused into the hippocampal CA3 subregion of 8-week-old mice. After 60 days, DpdtpA (100μM/mL effective circulating blood volume per day in mice) or normal saline was infused intravenously by caudal vein for 2 weeks, and then behavioral test and spine density were examined.
(b) Both overexpressing hTau and DpdtpA did not significantly change the escape latency for 5 days MWM training in mice (n=10 in each group).
(c-d) DpdtpA significantly improved hTau-induced spatial memory deficits evidenced by the decreased latency to the platform quadrant and the increased target crossings measured at day 7 by the removed hidden platform.
(e-f) Both overexpressing hTau and DpdtpA did not significantly change the motor function evidenced by the unchanged swimming speed detected at day 7.
(g-h) DpdtpA significantly improved hTau-induced cognitive deficit evidenced by the increased percentage of residence time in the new arm detected at 1 h and 24 h after Y-maze training.
(i-j) DpdtpA restored the hTau-induced reduction of hippocampal CA3 to CA1 synaptic transmission evidenced by the increased slope of fEPSP after HFS (n=3~5 successful recordings in each group).
(k-l) DpdtpA restored the hTau-induced reduction of dendritic spines in the hippocampal CA3 subset stained by Golgi stain (n=3~5 mice in each group).

Results

Figure 6. DpdtpA alleviates tau pathology and kinase/phosphate imbalance by inhibiting iron aggregation in mice.



(a-b) Intravenous administration of DpdtpA (100μM/mL effective circulating blood volume per day in mice) for 2 weeks attenuated AD-like tau hyperphosphorylation (AT8, Ser356, Thr205, Ser214, Thr231 sites) and accumulation (Tau5) measured by Western blotting.
(c-d) Intravenous administration of DpdtpA improved the hTau-induced imbalance of CaN and GSK3β measured by Western blotting.
(e) Intravenous administration of DpdtpA decreased the hTau-induced iron accumulation in hippocampus CA3 region detected by atomic absorption spectrometry.

Conclusion

The distinct effect of DpdtpA on iron metabolism suggested it may be a potential therapeutic drug for AD. In the current study, we further investigated the effect of the new iron chelator on memory loss induced by Tau overexpression. Expectedly, DpdtpA significantly attenuated learning and memory impairment induced by Tau aggregation in the hippocampus in mice. Our study provides definitive evidence for metal ion deposition induced by Tau pathology, not only Aβ, and highlights a new insight into iron chelation therapy in the progression of AD.