RXR agonist CNX-013-B2 has a potential to reduce Aβ deposition, increases clearance, along with reduced neuronal death in both N2a cells and astrocytes.

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Abstract

Objectives: Alzheimer's disease (AD) is a neurodegenerative process involving amyloid-β (Aβ) peptide deposition, neuroinflammation, and progressive memory loss. Aβ accumulation leads to the deposition into plaques and is believed to play a role in initiating the pathologic cascade leading to neuronal death. Cholesterol transport protein Apolipoprotein E along with ATP-binding cassette transporter A1 (ABCA1) plays an important role in the clearance of Aβ from the brain. It is known that RXR/LXR functions as a sensor of cellular cholesterol concentration and mediates cholesterol efflux by inducing transcription of key cholesterol shuffling vehicles, ABCA1 and ApoE. In this study, we report impact of a potent and selective RXR agonist CNX-013-B2 on Aβ deposition, clearance, neuroinflammation/stress and neuroprotection in both neuronal cells and astrocytes.

Methods: N2a cells and mouse astrocytes were used to study the impact on the expression of markers of different mechanisms that regulate Aβ deposition, clearance, neuroinflammation/stress and neuroprotection after treating the cells with high cholesterol.

Results: Treatment with CNX-013-B2, a selective small molecule rexinoid with an EC50 of 48 nM towards human RXRa resulted in a significant increase in cholesterol efflux along with 3-5 fold increase in both ABCA1 and ABCG1 gene expression. CNX-013-B2 increases mitochondrial biogenesis and activity along with increased expression of NMDR, PGC1a and FOXO1 which are known as neuroprotective markers. CNX-013-B2 reduces ROS levels, MCP1 and IL1b expression along with decrease in caspase3 levels.

Conclusions: RXR activation with CNX-013-B2 can reduce Aβ deposition and neuroinflammation/stress, can increase clearance and can act as a neuroprotective agent.

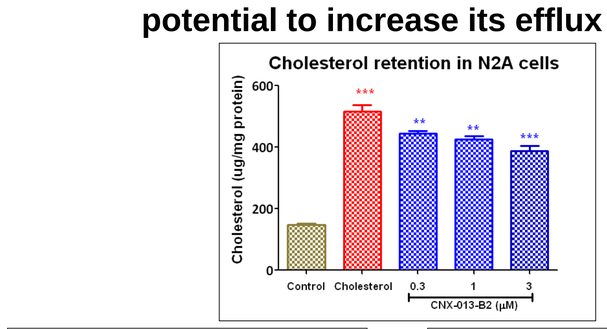
Materials and methods

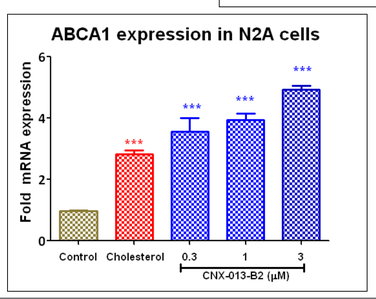
Mouse neuroblastoma (N2A, ATCC) cells were treated with cholesterol (320 µM) in MEM + 3 % FBS and incubated for 72h with and without CNX-013-B2. Media was replaced with fresh media once in 24h. Astrocytes (ScienCell) were pretreated with CNX-013-B2 for 24 h and challenged with LPS (100 ng/ml) in the presence or absence of the CNX-013-B2 for 18h. ROS estimation in astrocytes was performed after treating them with LPS (100 ng/ml) and PMA (10 nM) in the presence or absence of CNX-013-B2 for 18h. Cholesterol extraction from cells was done by hexane: Isopropanol (1:15) and quantified by Cholesterol FS reagent (Diasys). Relative gene expression studies were done by real time PCR. ROS were estimated using DCF dye. Released IL6 was measured using ELISA kit (R&D systems) and normalized with total protein content. Caspase3 release (Z-DEVD-R110 based kit, Invitrogen) and MTT assay were done after treating the cells with 80 µM cholesterol for 48h. For protein expression/modification studies, N2a cells were over expressed with human amyloid precursor protein (APP 695). Western blot followed by ECL was done to capture the signals with specific primary antibodies namely phospho mTOR, pTau, CyclinD and pAkt. Antibodies against beta Actin or GAPDH were used as internal controls. Statistical analysis was performed using unpaired students t test. (*p<0.05, **p<0.01, ***p<0.001).

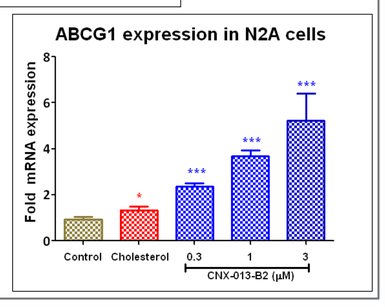
CNX-013-B2 profile

- It is a highly selective rexinoid.
- It does not activate RXR/RAR, RXR/AhR and RXR/PXR heterodimers
- EC50 is ~48nM towards RXRα, β and γ isoforms
- It has shown PPAR pan heterodimer activity
- It has a very good pharmacokinetic and dynamic properties

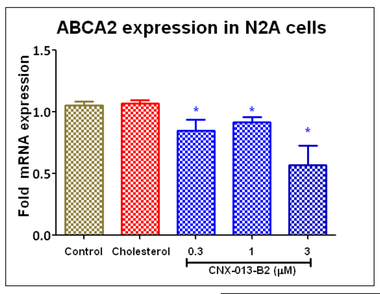
CNX-013-B2 reduces cholesterol accumulation and potential to increase its efflux

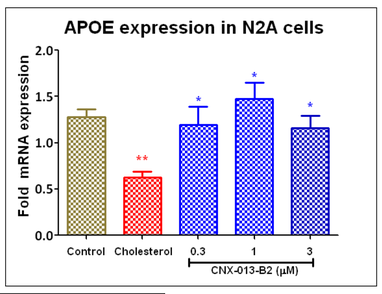


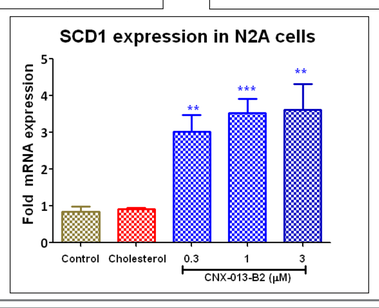




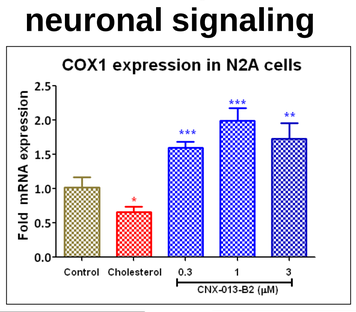
CNX-013-B2 has a potential to improve Aβ clearance

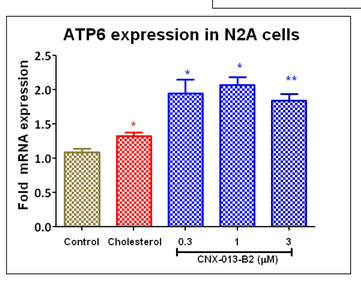


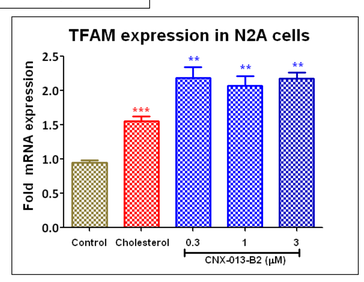




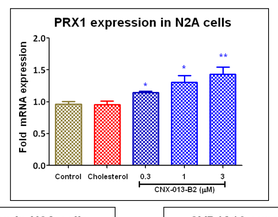
CNX-013-B2 improves mitochondrial function, health and hence has a potential to improve neuronal signaling

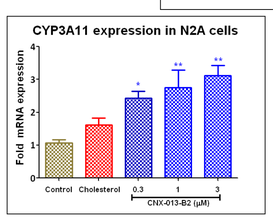


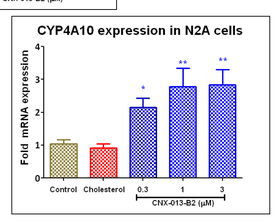




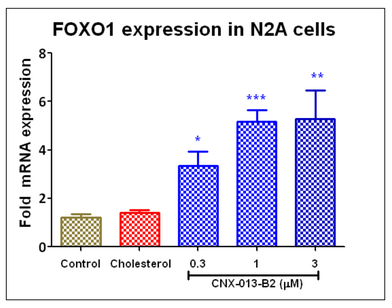
CNX-013-B2 increases markers of anti-oxidant and detoxification markers

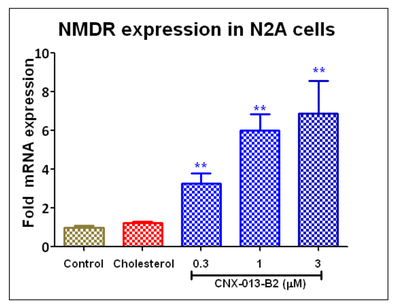


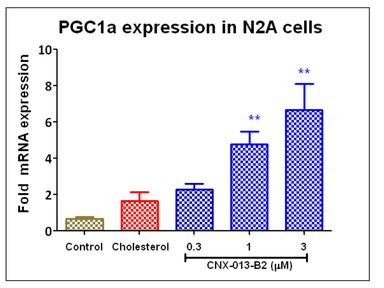




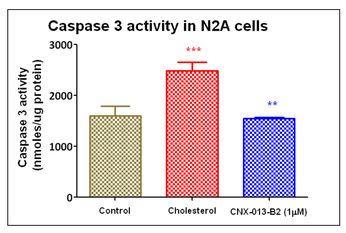
CNX-013-B2 has a potential enhance neuroprotection

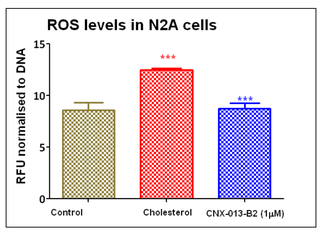


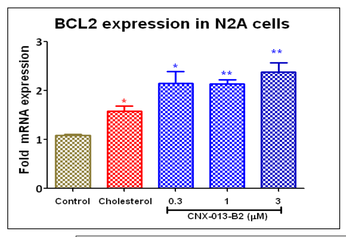


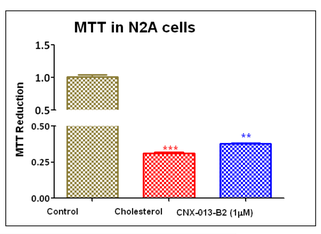


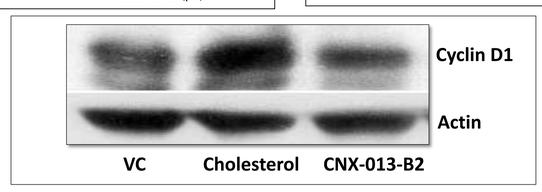
CNX-013-B2 has potential to reduce oxidative stress, inflammation, apoptosis and hence improve the neuronal health



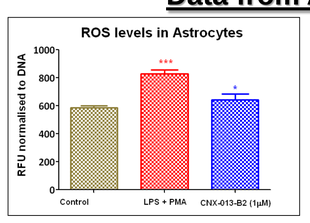


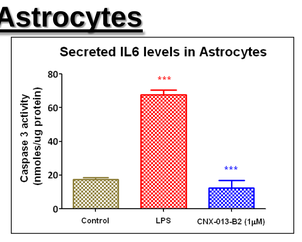


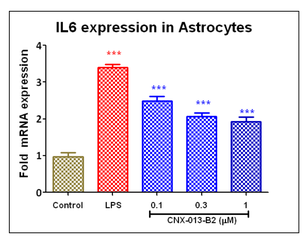


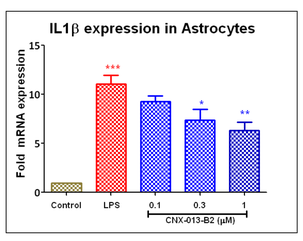


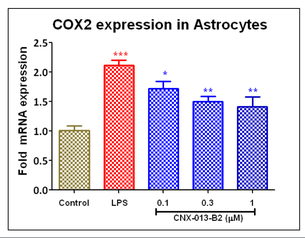
Data from Astrocytes

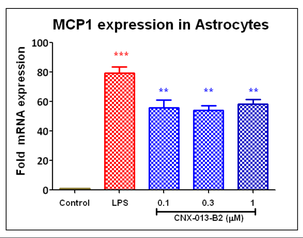




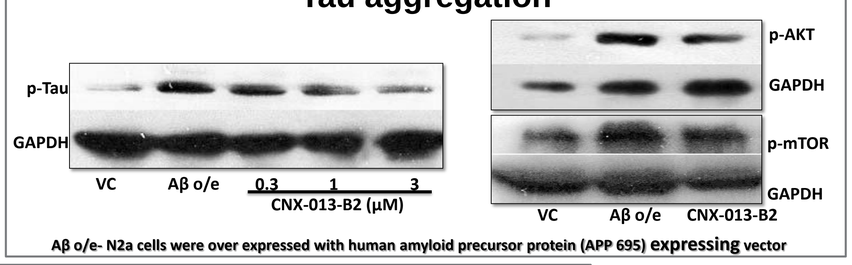








CNX-013-B2 has a potential to reduce Aβ-mediated Tau aggregation



Aβ o/e- N2a cells were over expressed with human amyloid precursor protein (APP 695) expressing vector

Conclusions

CNX-013-B2 has a potential to

- Decrease cholesterol accumulation and increase its efflux
- improve Aβ clearance
- Improve mitochondrial function, health and hence has a potential to improve neuronal signaling
- Enhance neuroprotection
- Improve neuronal health
- Decrease Tau aggregation

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