A Novel Therapeutic (NTRX-07) Targeting Neuroinflammation in Alzheimer's disease is Undergoing Phase I trials

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Abstract:

Mohamed Naguib, MSc, FCARCSI, MD and Tony Giordano, PhD, Cleveland Clinic, Cleveland, OH, USA

Background: Neuroinflammation plays a central role in the pathogenesis of Alzheimer's disease (AD). More than 25 genetic loci have been associated with the risk of developing late onset AD, and many of the linked genes are expressed in astrocytes, microglia, and oligodendrocytes. Reactive microglia and astrocytes express cannabinoid type 2 (CB2) receptors in post-mortem brains of AD patients and AD rodent models as well as in other neurodegenerative disorders. Activation of the CB2 receptor system results in inhibition of neuroinflammatory signaling pathways and restoration of normal microglial activity in AD and other neurodegenerative disorders. **Method:** We have developed a novel, blood brain barrier-permeable, and highly selective CB2 agonist that lacks psychoactivity, NTRX-07 (also known as MDA7), 1-[(3-benzyl-3-methyl-2,3-dihydro-1-benzofuran-6-yl) carbonyl] piperidine (Fig. 1), which will undergo Phase I trials in 2019 under an accepted Investigational New Drug Application. We have studied the effect of NTRX-07 on ameliorating the neuroinflammatory process, synaptic dysfunction, and cognitive impairment in APP/PS1 mice and in rats received bilateral microinjection of amyloid-beta (Ab_{1-40}) fibrils into the hippocampal CA1 area. **Result:** NTRX-07 treatment has been shown to i) restore physiological microglial activity (Fig. 2), ii) inhibit the production of inflammatory cytokines by modulating TLR4-NF-kB-MyD88 signal pathways (Fig. 3), iii) promote Aβ clearance (Fig. 4), and iv) restore synaptic plasticity, cognition, and memory in rodent models of AD (Figs. 5 and 6). **Conclusion:** Our findings suggest that NTRX-07 has a potential therapeutic effect in the setting of AD. Our clinical development program includes a single ascending dose, randomized, placebo controlled Phase Ia study with normal volunteers scheduled to begin in the Summer of 2019, which will be followed by a randomized, placebo controlled, multiple ascending dose, 28 days treatment Phase Ib study in AD subjects where CSF samples will be collected before and after treatment for biomarker analysis. The analysis of neuroinflammatory, Aß, tau and synaptic biomarkers will provide an indication of whether the effects of NTRX-07 observed in the rodent studies will translate to human AD subjects.

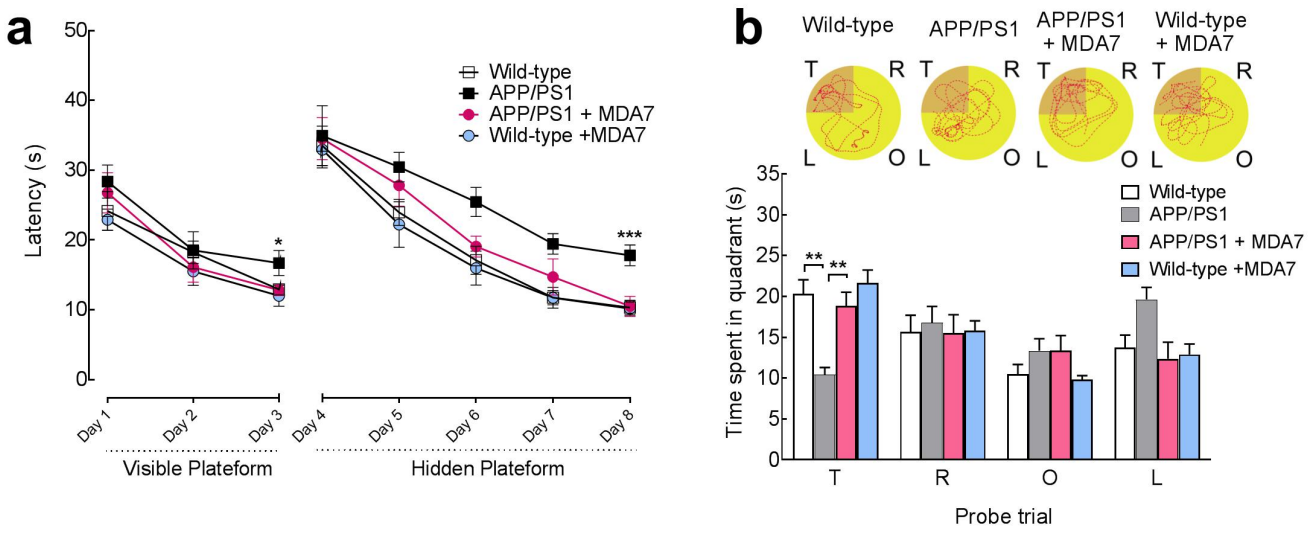

Fig. 6. Administration of NTRX-07 significantly attenuated impairment of memory performance in the Morris water maze test in APP/PS1 mice. (a) Significantly extended escape latencies in the Morris water maze were observed in APP/PS1 mice but not mice treated with MDA7; at days 1-3 (n = 10 in each group, effect of group [F3, 36 = 3.15, P = 0.04], effect of time [P < 0.0001], interaction between group and time [P = 0.88]) and at days 4-8 (n = 10 in each group, effect of group [F3, 36 = 9.75, P = 0.0001], effect of time [P<0.0001], interaction between group and time [P = 0.92]). (b) APP/PS1 mice but not those treated with MDA7 exhibited less time spent in the target quadrant (n = 10 in each group, F3, 36 = 11.5, P < 0.0001).

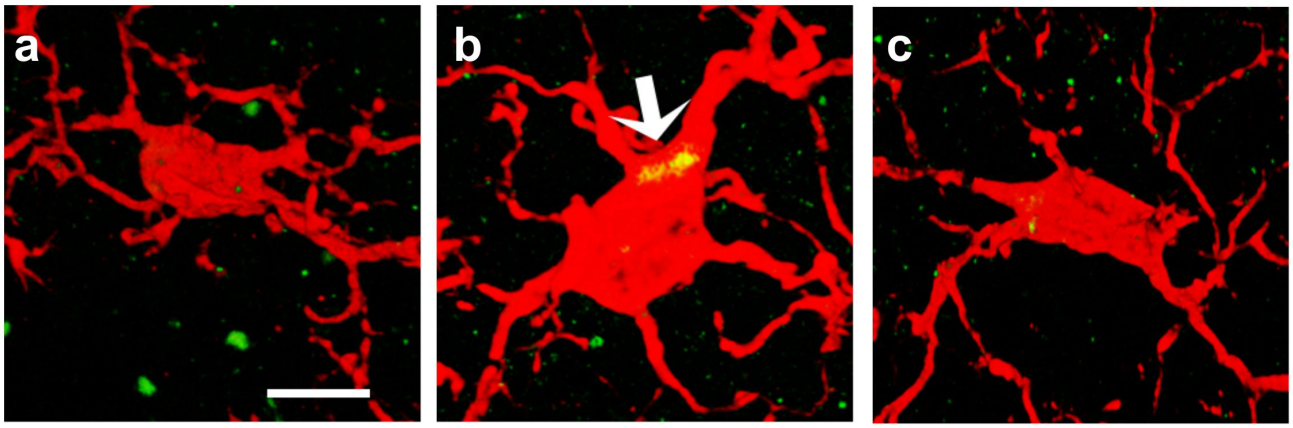

Fig. 2. Treatment with a selective CB2 agonist, NTRX-07, restored microglial function and normal morphology in an animal model of AD. No substantial cannabinoid type 2 (CB2) receptors expression is seen in the microglia from wild-type animal (a), but increased expression of CB2 is seen in reactive microglia derived from an animal model of AD (arrow, b). The figure depicts 3D immunofluorescence confocal images of the microglial marker ionized calcium binding adaptor molecule (1 lba1) (red) and the immunosignal of CB2 receptors (green) in microglia; the colocalization of CB2 and microglia is shown in yellow. Note the change from a highly branched and ramified morphology under normal physiological conditions (a) to an amoeboid form in the presence of neuroinflammation (b). (c) Treatment with NTRX-07 restored microglial function and normal morphology. Scale bar = 10 μm.

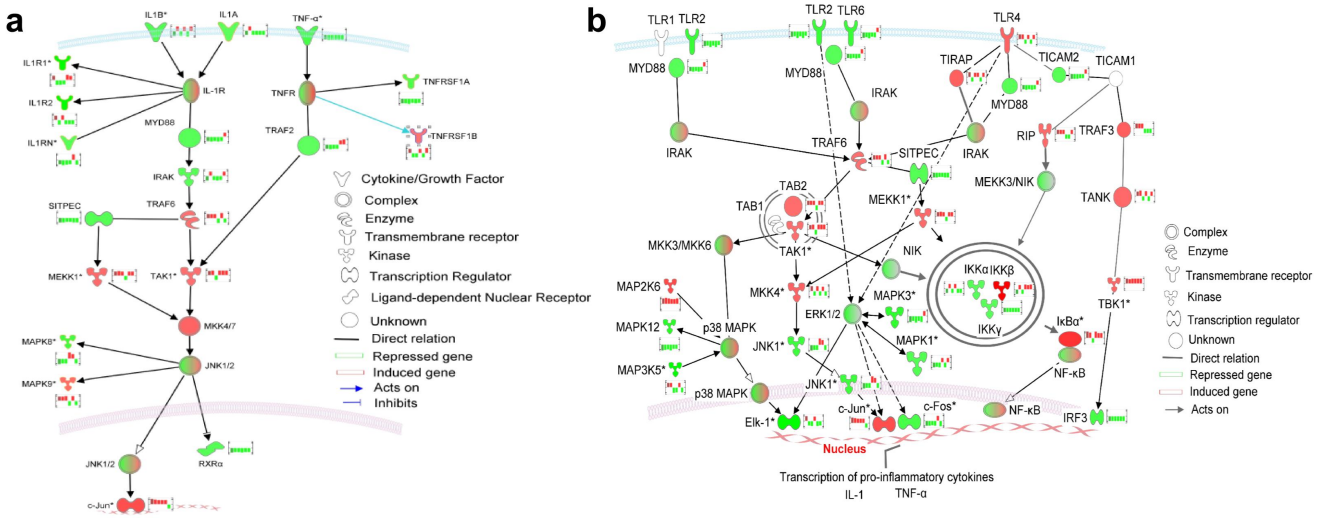

Fig. 3. (a) Modulation of IL-1 and TNF-α pathways by MDA7. Node brightness is proportional to the fold changes of gene expression. Color indicates up-regulated (red) and down-regulated (green) genes. In IL-1 pathway, a complex is formed including IL-1R-associated kinases (IRAK) and adapter protein MyD88. IRAK is rapidly phosphorylated and associates with TNF receptor-associated factor 6 (TRAF6), which is necessary for downstream IL-1-induced translocation of signaling molecules to the nucleus and ultimately leads to expression of genes that mediate inflammation. MDA7 may inhibit this signaling pathway. (b) Signaling cascades initiated via TLR2- and TLR4-dependent activation and its modulation by MDA7. Engagement of membrane TLR2 as a heterodimer with TLR1 or TLR6 leads to recruitment of MyD88 and interaction with TIR-domain-containing adaptor protein (TIRAP). Beneficial effects of MDA7 include modulation of general genes that will ultimately inhibit phosphorylation of IκB proteins by the IKKs and prevention of I B degradation. TLR4-MyD88-independent pathway activation involves signaling through the Toll-interleukin-1 receptor (TIR) adaptor TRIF (also known as TICAM1), TRIF-related adaptor molecule (TRAM; also known as TICAM2), TRAF3, receptor-interacting protein (RIP) and the transcription factor interferon regulatory factor 3 (IRF3).

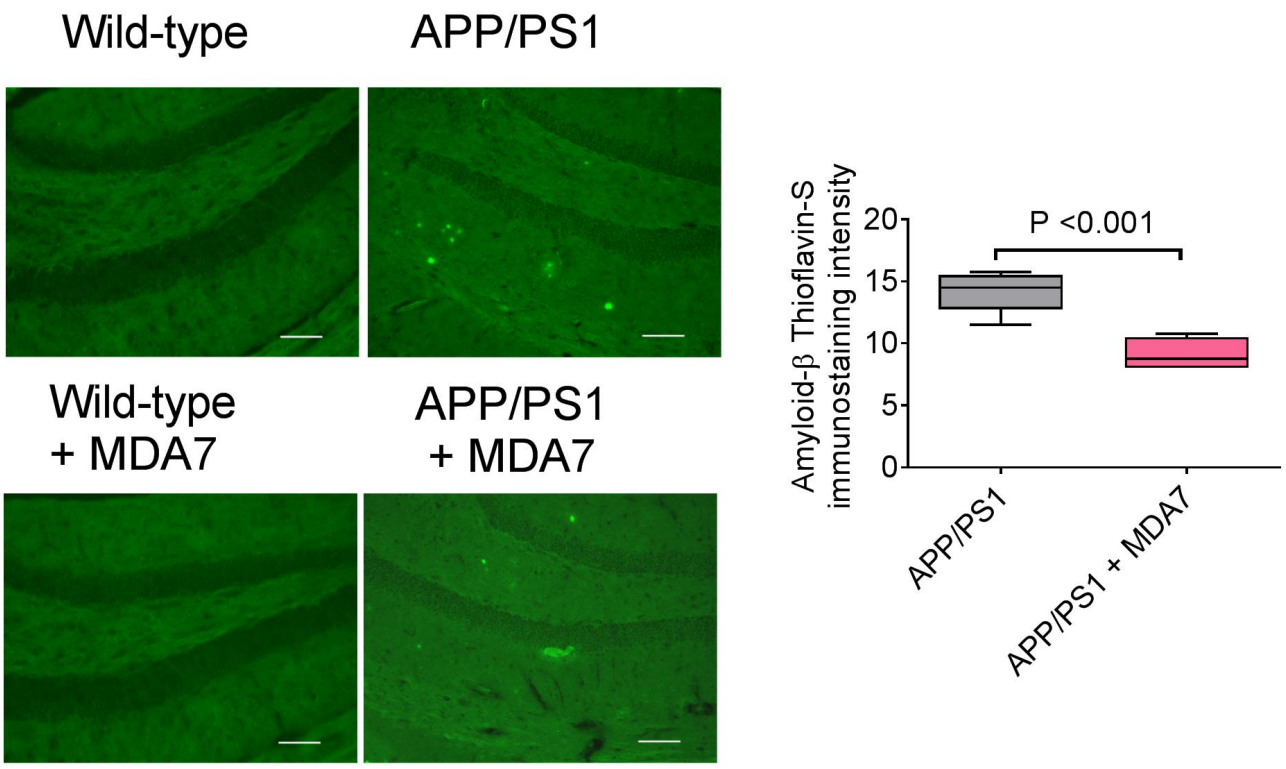

Fig. 4. NTRX-07 promotes Aβ plaque clearance in APP/PS1 mice. Immunofluorescence images of thioflavin S staining for Aβ plaques in the hippocampal DG areas in different groups. Administration of MDA7 significantly promoted Aβ plaque clearance (n= 20 sections from 5 mice per group, t = 5.39, two-tailed P <0.001). The total corrected cellular fluorescence (intensity) was calculated as the integrated density - area of selected cell × mean fluorescence of background readings. Box-and-whisker plots represent the interquartile range, minimum and maximum. Scale bar = 100 μm.

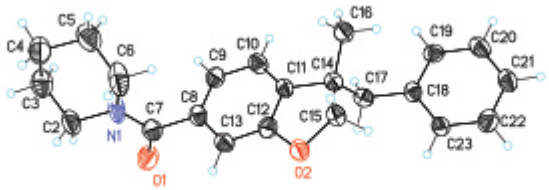

Fig 1. Structure of NTRX-07, 1-[(3-benzyl-3-methyl-2,3-dihydro-1-benzofuran-6-yl) carbonyl] piperidine.

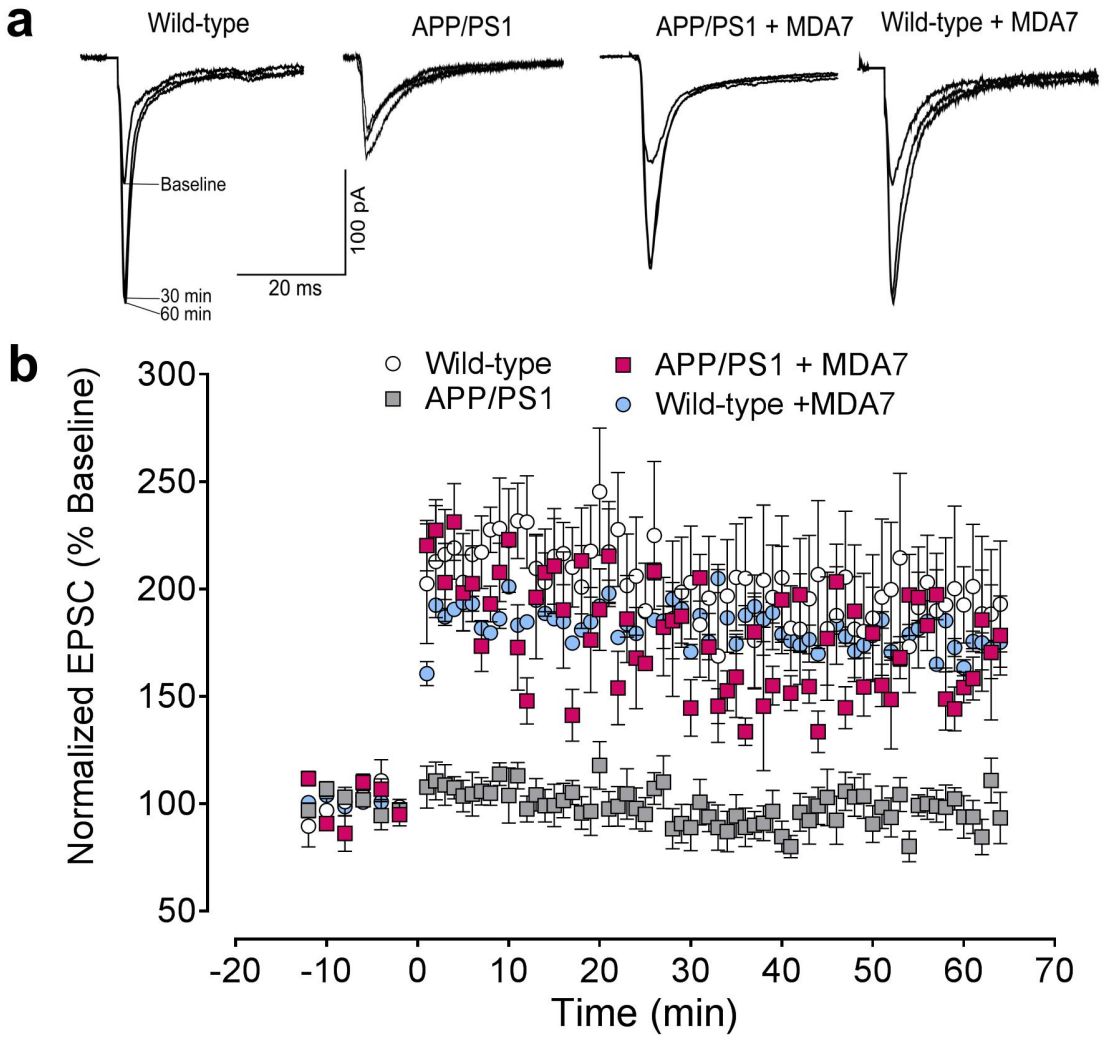

Fig. 5. Administration of NTRX-07 restored the long-term potentiation (LTP) in the hippocampal CA1 slices in APP/PS1 slides. LTP was induced by the electric stimuli on the Schaffer collateral-commissural fibers at 100 Hz for 1 second. (a) Representative traces to show the evoked EPSCs at baseline, 30, and 60 minutes after electric induction in all 4 groups. (b) Time course of the LTP induction in the hippocampal CA1 neurons in all 4 groups (n = 7,7,7,9 neurons from 4 to 5 mice per group, two-way ANOVA, effect of group [$F_{3,26} = 10.82$, $P = 0.001$], effect of time [$P = 0.004$], interaction between group and time [$P = 0.99$]). Data represent mean ± S.E.M.