

Therapeutic Effect of the Imidazoline I2 Ligand BU224 in a Model of Tauopathy

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Background

Current treatments for Alzheimer's disease (AD) are mainly focusing on managing symptoms or targeting amyloid- β with monoclonal antibodies, but these often show limited efficacy or have adverse effects.¹ Imidazoline I2 receptors are abundantly expressed throughout the brain and imidazoline agents have been shown to offer both neuroprotective and anti-inflammatory benefits.² As neuroinflammation is believed to play a key role in the progression of the pathology,³ imidazoline I2 receptor ligands can be considered as a potential novel treatment for AD.

Previous research from our lab has already demonstrated that treatment with the imidazoline I2 receptor ligands BU224 and CR4056 improved cognitive deficits and reduced neuroinflammation in the 5xFAD mouse model of AD without affecting amyloid pathology.^{4, 5} As tau pathology is also believed to contribute to the neurodegeneration seen in AD,⁶ this study aimed to investigate the therapeutic potential of BU224 in a model of tauopathy using the tau-P301S mouse model.

Hypothesis

Sub-chronic treatment with the imidazoline I2 receptor ligand, BU224, improves both behavioural and pathological deficits in the Tau-P301S mouse model.

Aim 1: To determine the effect of BU224 treatment on behaviour in Tau-P301S mice.

Aim 2: To investigate effects of BU224 treatment on tau pathology.

Aim 3: To investigate whether BU224 impacts other key pathological features of Alzheimer's Disease, including neuroinflammation and synaptic density.

Methodology

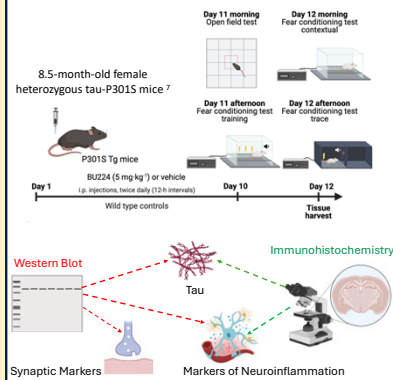


Table 1. Antibodies used in Western Blots.

Antibody	CP13	Tau-5	Synaptophysin	PSD-95	GFAP	GS	INOS	ApoE	Iba1	β -Actin
Host	Mouse	Mouse	Mouse	Rabbit	Rabbit	Rabbit	Rabbit	Mouse	Rabbit	Mouse
Target	P-Tau	Tau	Synaptophysin	PSD-95	GFAP	GS	INOS	ApoE	Iba1	β -Actin
Protein	at Ser202									
Molecular Weight (kDa)	64	64	38	95	50	45	130	35	17	42

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Behavioural Tests

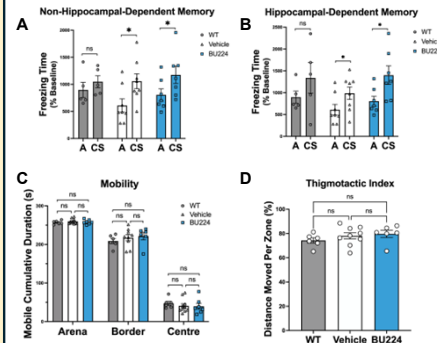


Figure 1. (A, B) Analysis of the fear conditioning test demonstrating the freezing time of the mice during 'A' (acclimatisation) and 'CS' (Conditioned Stimulus) bars represent mean $\pm$ SEM. (A) 'CS' refers to conditional tone which assesses non-hippocampal-dependent memory. (B) 'CS' refers to conditional trace which assesses hippocampal-dependent memory. (C) Analysis of mobility in the openfield test demonstrating the mobile cumulative duration of each group per zone in the arena. Bars represent mean $\pm$ SEM. (D) Demonstrates the thigmotactic index which is a measure of the % of distance moved that occurs in the 'Border' zone during the openfield test. ns = Not significant; * $p < 0.05$.

BU224 Increases Synaptic Density in the Cortex

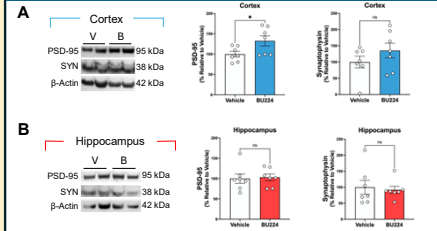


Figure 2. (A, B) Shows a representative Western Blot (WB) for the synaptic density markers PSD-95 and SYN (synaptophysin), and the loading control β -Actin. 'V' denotes Vehicle and 'B' denotes BU224. Also shown are graphs analysing the WB images for both PSD-95 and synaptophysin. Bars represent the mean $\pm$ SEM of the % relative to the vehicle group. (A) Cortex. (B) Hippocampus. ns = Not significant; * $p < 0.05$.

BU224 Reduces GFAP and Increases ApoE Expression

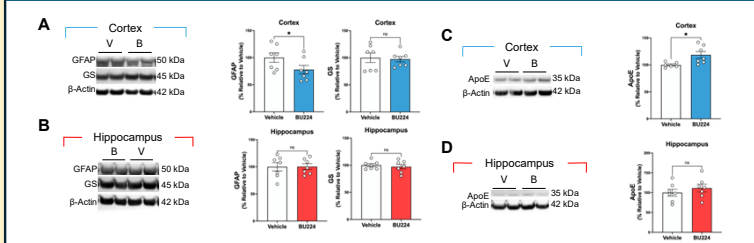


Figure 3. (A-D) Show representative Western blots (WB). 'V' denotes Vehicle and 'B' denotes BU224. Also shown are graphs analysing the WB images. Bars represent the mean $\pm$ SEM of the % relative to the Vehicle group. (A) Results for GFAP and GS (glutamine synthetase) in the cortex. (B) Results for GFAP and GS in the hippocampus. (C) Results for ApoE in the cortex. (D) Results for ApoE in the hippocampus. ns = Not significant; * $p < 0.05$.

BU224 Does Not Affect Tau Pathology

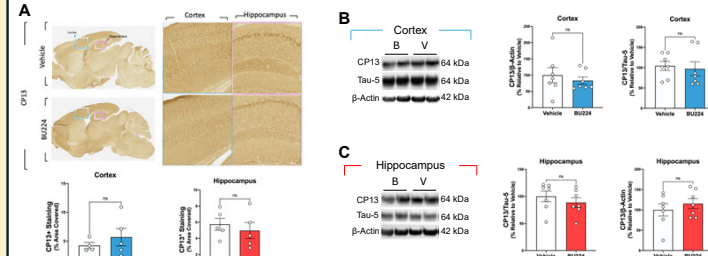


Figure 3. (A) Representative image from immunohistochemistry for CP13 in the cortex and hippocampus and analysis of the % area covered with CP13⁺ staining. Bars represent mean $\pm$ SEM. (B, C) Shows a representative Western Blot (WB) for the p-Tau marker CP13, the total tau marker Tau-5 and the loading control β -Actin. 'V' denotes Vehicle and 'B' denotes BU224. Also shown are graphs analysing the WB images for both CP13 and Tau-5. Bars represent the mean $\pm$ SEM of the % relative to the vehicle group. (B) Cortex. (C) Hippocampus. ns = Not significant; * $p < 0.05$.

BU224 treatment Does Not Alter Microglia Density and Neuroinflammatory Markers

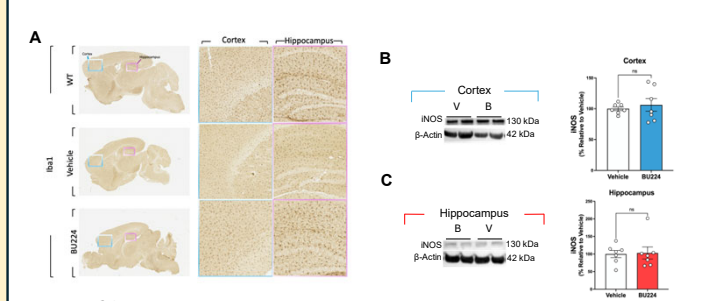


Figure 4. (A) Representative image from immunohistochemistry for Iba1 in the cortex and hippocampus and analysis of the process length. Bars represent mean $\pm$ SEM. (B, C) Shows representative Western Blots (WB) for Iba1 and the loading control β -Actin. 'V' denotes Vehicle and 'B' denotes BU224. Also shown are graphs analysing the WB images for Iba1 and INOS. Bars represent the mean $\pm$ SEM of the % relative to the vehicle group. (B) Cortex. (C) Hippocampus. ns = Not significant.

Conclusions

- BU224 increases synaptic density in tau-P301S mice.
- BU224 reduces GFAP expression, indicating decreased reactive astrocyte density.
- BU224 treatment resulted in increased ApoE expression.
- No significant changes were observed in motor or cognitive behaviour.
- No changes in p-tau pathology were detected following treatment or in microglia activation.
- These findings suggest BU224 exerts neuroprotective effects independent of direct p-Tau modification.

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