Cisplatin and the Mu Opioid Receptor Antagonist Methylnaltrexone Inhibit Neurite Growth in Cultured Trigeminal Neurons

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Introduction

Opioids are a common class of drugs that treat moderate to severe chronic pain. Cancer patients with head and neck squamous cell carcinoma present with the highest prevalence of pain, requiring the administration of opioids for pain management. Pain is exacerbated in these patients due to perineural invasion (PNI), the process through which cancer cells invade the surrounding nerves' perineural spaces[1]. Despite the benefits of pain suppression, opioids have been investigated for their role in cancer progression, metastasis, and recurrence through the overexpression and activation of Mu-opioid receptor (MOR) [2]. As a result, MOR may be a potential therapeutic target in cancer treatment. Low doses of the Mu Opioid receptor antagonist, methylnaltrexone (MTNX), has shown to inhibit head and neck squamous cell carcinoma growth in vitro and in vivo[3]. However, the mechanism with which MTNX decreases tumor growth has yet to be elucidated. We aimed to evaluate MTNX and Cisplatin's effect on the functionality of trigeminal neurons, implicated in head and neck tumors, by measuring neurite outgrowth. We hypothesize that decreased neurite growth may reduce the cross talk between cancer cells necessary for PNI and tumor growth.

Methods

Trigeminal Ganglia Cell Culture

- Athymic nude mice were deeply anesthetized, and the trigeminal ganglia were excised and placed in a culture dish with trypsin and collagenase for digestion
- After overnight incubation, cells were then treated with the following conditions: 1.25 uM cisplatin, 1000 nM methylnaltrexone, or 1.25 uM cisplatin and 1000 nM methylnaltrexone. Cells were cultured with Cisplatin for six hours only, while MTNX was present continually to replicate the dosing regimen used in tumor-beating mouse experiments

Immunocytochemistry

- Cells were fixed with 4% paraformaldehyde and blocked with 5% normal donkey serum and 0.2% Triton X-100 in 1X PBS for 1 hr
- Plates were incubated overnight with 1:1000 FITC-conjugated anti-beta III tubulin antibody
- Slides were mounted and viewed with a fluorescence microscope. All images were taken using the same acquisition parameters, and image analyses were performed by experimenters blinded to treatment conditions

Stereological Analysis

- Image template consisting of seven evenly spaced test lines in a square format overlaid onto the neuron images
- Number of somas and intersections per image were counted manually
- Neurite growth length calculated using the formula Length= $(78.5 \times i)/s$, where i represents the number of intersections and s is the total number of neuron somas present in the image.

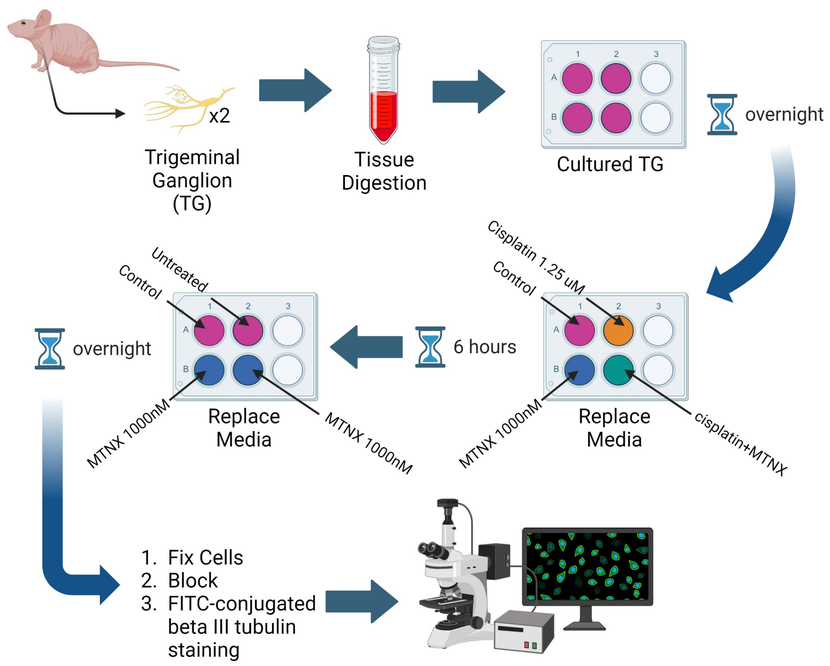

Fig. 1 Experimental protocol for neurite growth experiments.

Results

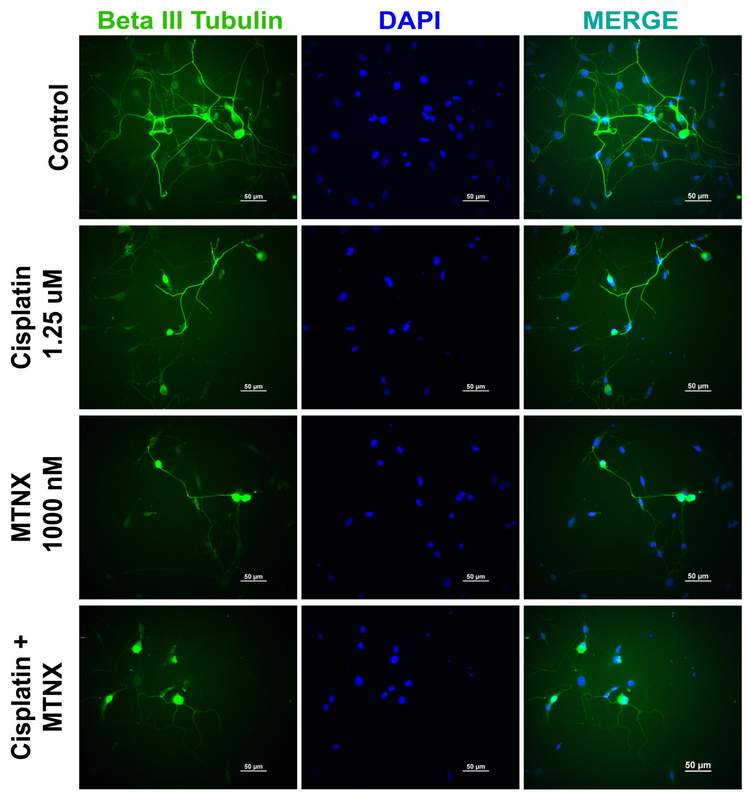

Fig. 2 (Left): Beta III Tubulin and nuclear DAPI staining in various treatment conditions show neuronal somas and associated axonal and dendritic growth. Compared to control cells, all three treatment groups displayed decreased neurite outgrowth. Scale bar = 50 µm

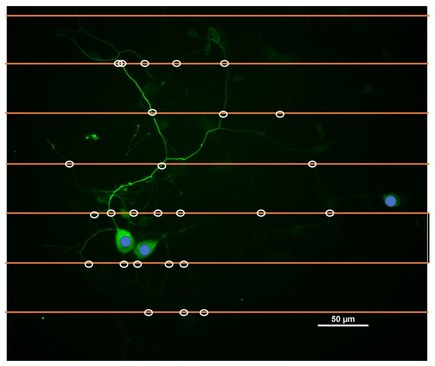

Fig. 3 (Above): A representative example of an image overlaid with the test line grid (orange) for neurite growth assessment. Neuron somas are marked by blue circles and neurite crosses are marked in white circles. Scale bar = 50 µm

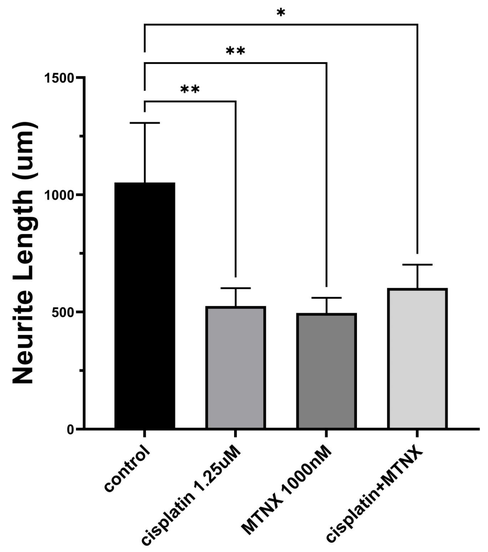

Fig. 4 There was a significant overall effect for the experimental group on neurite lengths ($F_{3,87} = 3.1$, $p<.05$). Post-hoc tests revealed that treatment with 1.25 uM cisplatin, 1000 nM methylnaltrexone, or 1.25 uM cisplatin and 1000 nM methylnaltrexone was associated with significantly lower neurite outgrowth. There were no significant differences among the three treatment groups.

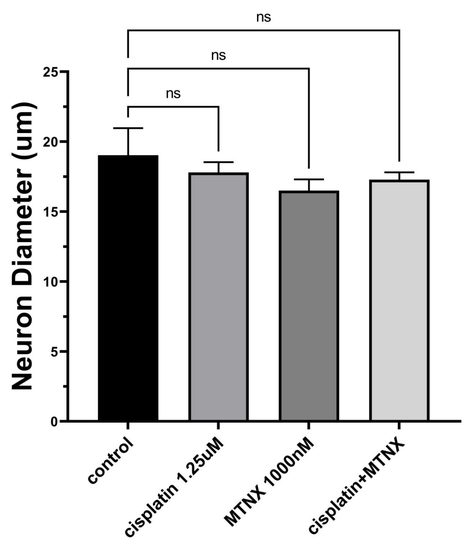

Fig. 5 There was not a significant overall effect for experimental group on neuron diameter ($F_{3,87} = 1.8$, n.s.). Mean neuron diameter did not differ among the groups, and the proportion of small-diameter neurons (<27um) was similar among control (95.5%), 1.25 uM cisplatin (93.9%), 1000 nM methylnaltrexone (92.9%), and 1.25 uM cisplatin and 1000 nM methylnaltrexone (95.0%).

Conclusions

In vitro experiments demonstrated that MTNX and Cisplatin decrease neurite outgrowth, suggesting a possible decrease in crosstalk and subsequent decreases in perineural invasion contributing to tumor growth. Administration of opioid antagonists in conjunction with chemotherapy drugs may inhibit cancer metastasis and lead to decreased pain outcomes for patients, ultimately reducing the opioid burden.

References

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