

Regenerative Capacity of Cultured Neurons Following Photodynamic Therapy in a 3D Collagen Gel Culture System

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INTRODUCTION: Photodynamic therapy (PDT) is a promising treatment modality for cancer which involves administration of a photosensitising agent that can be activated subsequently within a patient's cells, resulting in cell death from oxidative damage. Peripheral nerve sparing has been reported following PDT with the photosensitiser *meta*-(tetra-hydroxyphenyl) chlorin (mTHPC) [1 & 2]. Dorsal root ganglia (DRG) neurons have been shown to be relatively insensitive to mTHPC-PDT doses that killed other cell types in a 3D collagen culture system [3]. The aim here was to determine the extent to which 'surviving' neurons were able to sprout neurites as an indication of functional recovery following PDT.

METHODS: Mixed cultures of dissociated neurons and satellite glia were prepared from the DRGs of 250-300 g rats and maintained within thin 3D collagen gels for 4 days. Cultures were exposed to 4 and 10 $\mu\text{g/ml}$ mTHPC for 4 h, then illuminated at a fluence rate of 1.6 mW/cm^2 for 10 min, giving a total light dose of 1 J/cm^2 [3]. After a latency period of 24 h, during which time a substantial number of satellite glia died, surviving neurons were extracted by digesting gels with 0.125 % collagenase for 30 min. PDT treated cells were collected and combined with untreated satellite glia then further cultured in monolayer on 20 $\mu\text{g/ml}$ PLL-coated 19 mm diameter coverslips for 2 days. The ratio of $\beta\text{III-tubulin}$ immunoreactive neurons with or without new neurite growth, and the lengths of neurites were assessed using fluorescence microscopy and digital image analysis.

RESULTS: Fig 1 shows the proportion of neuronal cell bodies that showed neurite growth. Neurons treated with 3 & 4 $\mu\text{g/ml}$ mTHPC-PDT showed no difference to untreated and light only controls in their ability to produce neurites. Fig 2 shows length of neurite outgrowth was not significantly different between control and light only and 3 & 4 $\mu\text{g/ml}$ mTHPC-PDT treated cells. Only 10 $\mu\text{g/ml}$ mTHPC-PDT treated cells showed significantly reduced neurite lengths in comparison to light only control (* $P < 0.05$; 1-way ANOVA with Dunnett's post test).

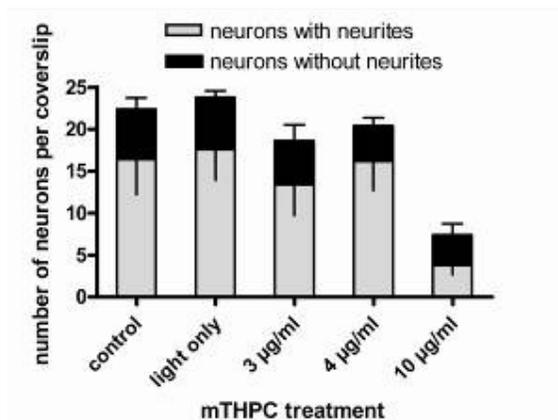


Fig. 1: Neurons showing neurite outgrowth

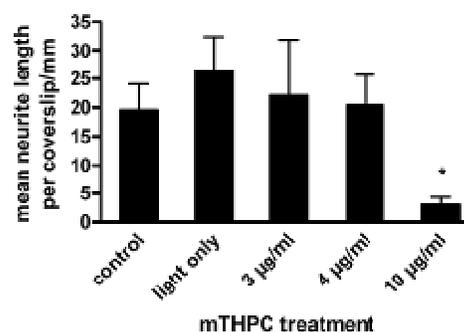


Fig 2: Length of neurite outgrowth

DISCUSSION & CONCLUSIONS: The ability of neurons treated with mTHPC-PDT to extend neurites in this model system acts as an indication that they remain functionally active in terms of regenerative capacity. This is an important finding since identifying PDT conditions that enable neurons to survive while tumor cells are destroyed may be of particular benefit for treating cancers within or adjacent to the nervous system.

REFERENCES: ¹C.M. Moore, T.R. Nathan, W.R. Lees et al. (2006) *Lasers Surg Med* 38(5):356-63. ²CS Betz, HR Jäger HR, et al. (2007) *Lasers Surg Med* 39(7):571-82. ³K.E. Wright, A J MacRobert, & J B Phillips (2008) *European Cells and Materials* Vol. 16. Suppl. 3:13.

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