Spinal Cord Explants *in vitro*: Effects of Static Magnetic Fields (SMF) and ara-C on Neurite Growth

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INTRODUCTION

We have reported that static magnetic fields (SMF) of 450 G or 900 G stimulate neurite outgrowth from dorsal root ganglia *in vitro*. To determine whether spinal cord segments would also respond to SMF was a logical next step, since there is now active interest in promoting regeneration of spinal cord. Ara-C (cytosine arabinoside), an anti-tumor agent, was included in these experiments since it inhibits DNA synthesis in non-neuronal cells whose overgrowth retards neurite growth (Sisken et al, 1985).

MATERIALS AND METHODS

8-9 day chick embryo spinal cord was sliced into approximately 100 μm thick pieces and set onto cooled 60 mm Falcon culture dishes containing a layer of Matrigel (Collaborative Res., Bedford, MA) diluted 1:3 with Neurobasal Medium (Gibco, N.Y.). Then the dishes were placed in a 37 °C incubator for 2 hrs. to "gel" the Matrigel and trap the tissue in the Matrigel. Equal portions of Neurobasal Medium containing B27 (Gibco) and penicillin and streptomycin, and Dulbecco Medium containing fetal calf serum and antibiotics were added to each dish. The dishes were placed on 60 mm diameter 900 gauss magnets (Magnetherapy, Inc, FL) for 1 hour, or on dummy magnets (no magnetic field) for 1 hour. Ara-C (25 μg/ml) was added to half of all dishes, and saline to the other half. Both control and experimental dishes were exposed to dummy magnets or 900 gauss magnets again for the following 2 days. The cultures were fixed in 4 % formalin after 5 days of growth *in vitro*.

RESULTS:

Our objective was to determine the amount of neurite growth occurring around the circumference of the spinal cord slice; this was done by dividing the spinal cord explant into quadrants. Neurite growth in these explants consisted of many long neurites (up to 5 mm) and was quantified first by assessing whether it was present in the following manner: in all 4 quadrants ("all"), in 3/4, 1/2, 1/4, or no quadrants ("none"). Only explants growing in the inner (35 mm) section of the culture dish were assessed. The majority of the explants grown without ara-C had no neurite outgrowth and displayed only a halo of non-neuronal cells surrounding the original spinal cord slice. About 20% had neurite growth in 1/4 of the quadrants, and 10% in 1/2 the quadrants. When ara-C was added to the explants, a large number of long neurites were observed emanating from the explant in unexposed dishes with 37% in 3/4 of the explant and 12% in all quadrants of the explant; 25% of the explants had no neurite outgrowth.

In dishes treated with ara-C and 900 G SMF the frequency of growth in all quadrants increased to 45%, 27% of which were in 3/4 of the quadrants. All explants in the SMF-Ara C group had some neurites and none were completely devoid of neurites.

DISCUSSION

Determination of the amount of growth from all quadrants was an important criteria in these studies. In future work we will measure the length and number of neurites to quantify the extent of this stimulation. Although this is work in progress, our initial results indicate some measure of stimulation of regenerative growth with high SMF of 900 Gauss. This stimulation occurs only in the presence of an agent such as ara-C that deters overgrowth of neurites by non-neuronal glial cells.

REFERENCES

[1] B.F. Sisken, M. Kanje, G. Lundborg and W. Kurtz, "Pulsed electromagnetic fields stimulate nerve regeneration *in vitro* and *in vitro*," *Restorative Neurology and Neuroscience* , vol. 1, pp. 303-309, 1990.

[2] B. Greenebaum, C. Sutton, M. Subramanian, J. Vadula, J.H. Battocletti, T. Swiontek, J. DeKeyser, and B.F. Sisken, "Effects of pulsed magnetic fields on neurite outgrowth from chick embryo ganglia," *Bioelectromagnetics*, vol. 17., pp. 293-302, 1996.

[3] Supported in part by Magnetherapy Inc., Riviera Beach, FL 33404