Neurotoxicity Screening Test for Deep Brain Stimulation Leads

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Statement of Purpose: Deep Brain Stimulation (DBS) is an approved therapy indicated for the treatment of symptoms associated with Essential Tremor and Parkinson's disease. The potential for application of this technology and variations of it in other disease states and other regions of the brain is rapidly increasing[1].

The DBS lead consists of a stimulation end, a terminal end and a lead body. Both the lead body and the stimulation end are exposed to cerebrospinal fluid and brain tissue during normal use. The stimulation end is comprised of four 90% Platinum/10% Iridium electrodes and 55D Aromatic Polycarbonate Polyurethane (55D Bionate) spacers. The lead body is made of 80A Aromatic Polycarbonate Polyurethane (80A Bionate). Each of these materials has been thoroughly tested per ISO and FDA standards. Because neither of these standards specifically addresses neurotoxicity, the next step in the screening process is typically large animal testing. In order to expedite time to human trials and to minimize expense and large animal sacrifice, an alternative method was developed to screen these materials for any human neurotoxic potential. The screening method consisted of cytotoxicity and cell viability tests of human cerebellar, cortical and glial cells using two mediums in contact with leachates extracted from the DBS leads.

Methods: Extraction Conditions – For these tests, a lead, negative control and positive control were extracted in an appropriate form of Eagle's Medium for 24 hours at 37°C and saline for 72 hours at 50°C. All test and control articles were extracted at a ratio of $60cm^2$: 20mL.

Cytotoxicity Test Procedure - Following the 24 and 72 hour extraction periods, the growth media from triplicate $10cm^2$ wells containing cell lines HTB-186, CRL-2366 and CRL-10442 (American Type Culture Collection, Manassas, VA) were decanted and replaced with 2mL of test article or control sample extract for media extraction and 2mL of diluted test article for saline extraction. All cell cultures were then incubated for 48 hours at 37°C in a humidified incubator with 5% CO_2. After 24 and 48 hours of incubation, all cells were examined under an inverted light microscope with 100X magnification. The results of the examination were scored according to ANSI/AMMI/ISO 10993-5:1999[2]. The average score for the triplicate test wells at the 48-hour endpoint was used to determine the cytotoxic response. The average score for the triplicate controls at the 48-hour endpoint was used to compare the results.

Cell Proliferation Assay Test Procedure - 100µL of cell suspensions containing varying amounts of cells, as determined by microscopic observation using a hemocytometer, were pipetted into 96 well cell culture plates in duplicate and then 20µL of the Cell Titer 96® AQueous One Solution Reagent (Promega, Madison, WI) was added to each well. Plates were incubated at 37°C in a humidified incubator with 5% CO_2. After 1, 2, 3 and 4 hours of incubation, the absorbance at 490nm was measured using a 96-well plate reader and the average absorbance was calculated and linear regression analysis was conducted to determine the effect of cell number on absorbance. 50µL of a cell suspension with a concentration of cells in the linear range of the assay described above was transferred into three 96 well cell culture plates with 24 wells/plate. 50µL of extracts from the lead and controls were added in triplicate. The plates were then incubated at 37°C in a humidified incubator with 5% CO_2. One plate per sample group (lead, positive control and negative control) was removed at each time interval (24, 48 and 72 hours). 20µL of the Cell Titer 96® AQueous One Solution Reagent was then added to each well. Again, the plates were incubated at 37°C in a humidified incubator with 5% CO_2 for 3 hours and 20 minutes. After the final incubation, the absorbance was measured at 490nm using a 96-well plate reader.

Results / Discussion: Cytotoxicity Test Results – After 24 and 48 hours of incubation, the cerebellar, cortical and glial cells exposed to both Eagle's Medium and saline displayed discrete intracytoplasmic granules and no cell lysis. Per ANSI/AMMI/ISO 10993-5:1999, these results translate to a score of 0, which is interpreted as a non-cytotoxic response.

Cell Proliferation Assay Test Results – After 1, 2, 3 and 4 hours of incubation, the cell viability of the cerebellar, cortical and glial cells was not affected by the presence of the lead extracts in both Eagle's Medium and saline when compared to the viability of the positive and negative controls extracted in the same media.

Conclusions: Neuronal cell specific cytotoxicity testing was conducted on three cell lines with results supporting safety of the DBS lead in human brain tissue. Testing was conducted in two separate growth environments: saline and Eagle's Medium. Extractions were made in saline and medium extraction wells from 3 intact, sterile test articles acquired from sterile finished product stock. Two neurotoxicity tests were conducted: the qualitative ISO Cytotoxicity Elution Test and the quantitative Cell Titer 96® AQueous One Solution Cell Proliferation Assay Test. None of the testing induced cytotoxic effect on human brain tissue by the DBS lead. In combination with ISO-10993 biological and materials characterization testing, human cell line in-vitro toxicity testing provides an economical, short duration, non-animal sacrifice means of screening for safety for human implantation.

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[1] Gross RE. Expert Rev Neurother. 2004 May;4(3):465-78

[2] Biological evaluation of medical devices–Part 5:Tests for in vitro cytotoxicity; ANSI/AMMI/ISO 10993-5:1999in AMMI Standards and Recommended Practices, Vol. 4S2 (2000)