PRELIMINARY STUDY REPORT

THE IN VITRO EFFECTS OF MEANDROTHERAPY ON SURVIVAL AND REPLICATION OF B16F10 MURINE MELANOMA CELLS VS. NORMAL RAT RENAL MESANGIAL CELLS

The study has been performed at the premises of Nephrology Research Laboratory,

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Experimental Design

Objectives and Aims of the experiment:

To test the hypothesis presented by Dr. Kutushov that the device (further addressed to as the meandrotherapy device), invented by him and his colleagues, is capable of decreasing survival and preventing proliferation of cultured tumour cells but sparing the survival and proliferation of normal tissue cells.

Methods and Study protocol:

1. Cell isolation and culture.

****Murine B16F10 melanoma cell line*** was initially purchased from ATCC (American Type Cell Collection**,** USA) and further subcultured at the premises of Nephrology Research Laboratory according to the instructions of the manufacturer.

****Rat renal mesangial cells*** were isolated from embryonic metanephroi of foetuses excised from Sprague-Dawley female rats at day 14 of pregnancy, for a different experiment unrelated to the present study. The cells were subcultured and purified by standard procedures used in our laboratory, and after the completion of cell differentiation to mesangial phenotype, two culture flasks containing such mesangial cells were allocated, to be used in Dr. Kutushov's experimentations.

2. Cell treatment.

At a day randomly chosen by Dr. Kutushov, two flasks containing murine B16F10 melanoma cells and two flasks containing rat renal mesangial cells were repeatedly washed out from their culture medium. Two millilitres of fresh medium supplemented with 10% bovine calf serum were placed in each flask.

One flask of each pair was personally treated by Dr. Kutushov, using the meandrotherapy device at a regimen that was tentatively chosen (20minutes, at a frequency of 22.5 hertz). The second flask of each pair remained in the meantime within the biologic hood. The hood was about 0.5m far from the workbench where the meandrotherapy device was placed. After the treatment, all four flasks were simultaneously treated with ^{3}H-Thymidine, 50μCi/ml (i.e.100 μCi/flask), placed back to the incubator and cultured for 24h at standard conditions for tissue culture (37ºC, 95% humidity, 5% CO_2).

Following 24h, the flasks were removed from the incubator. The cell medium was allocated and the cultures were gently washed three times by phosphate-buffered saline (PBS, pH7.3). The cells were stained with hematoxyline and analysed using the light inverted microscope. The microphotographs were taken using the Olympus CKX 41 microscope camera supplemented with CMS-2-M system as a part of Advanced Measurement Systems, Ltd (Israel).

The cells were then collected from the flask bottoms, placed in polystyrene vials containing a scintillalion cocktail based on ortho-toluidine, and ^{3}H-Thymidine incorporation was assessed in a β-Counter (Packard, USA).

Results and Conclusions

The microphotographs of the cells are attached to the report.

Cell survival

*Both B16F10 melanoma cultures, the control and the treated one, demonstrate visible signs of distress: the cells are round, detached from the flask bottoms and highly agglutinate, forming large clusters. Apoptotic cells (shrinking, losing their cytoplasm and containing condensed chromatine) are detectable mainly at the margins of the clusters. With respect to these signs, we could not see any difference between the treated and untreated cells.

* Microphotographs of the non-malignant mesangial cell cultures show that these cells, regardless of the treatment, haven't lost their ability to attach to the flask bottoms. Both cell cultures, the control and the treated one, contain pro-apoptotic cells in amounts not exceeding the normal range of 0.5%-1.0% per microscopic field. Apparently, treated cells demonstrate lower cell density than the untreated controls. Probably, it may be explained by higher rate of cell loss immediately after the treatment, in which case the cell remnants could be floating in the culture medium and then washed out, together with the excessive radioactive material, leaving the attached cells at lower density. However, this assumption can be proved only by additional experiments involving total cell protein measurements, which have not been included in the protocol of the present study.

Cell proliferation

All cell cultures, treated as well as untreated, appear to become quiescent, showing negligible levels of 3H-Thymidine incorporation. Their radioactive count per minute (CPM) readings were extremely low (20-30 CPM only) and non-distinguishable from the readings of unspecific binding.

We, in the lab, possess no actual knowledge concerning the theoretical background of the invention or the technical background of the tested device. Thereby, we are not able to draw any conclusions from the results herein presented. Yet, the explanation suggested by Dr. Kutushov might be plausible. According to this explanation, the tentatively chosen operating regime of the tested device and/or the time of treatment might have been inadequate (too powerful?) for cell cultures. Thereby, the radius and/or the magnitude of the spreading waves affected the survival and spontaneous proliferation rates of normal mesangial as well as malignant B16F10 cell cultures present in the room, regardless of their closeness to the device.

Figures

Rat mesangial cell vs. B16F10 murine melanoma cell cultures

A –Cells treated with the meandrotherapy device for 20 minutes, at a frequency of 22.5 hertz.

B – Untreated control cells.