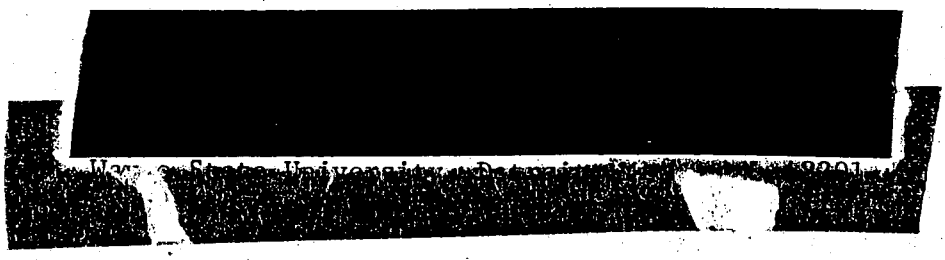
BE-12

MICROWAVE EFFECTS ON HUMAN COLONY FORMING MARROW CELLS



We examined the effects of 2450 MHz CW microwaves on human colony forming cells of the neutrophil lineage. Marrow cells from 3 patients with acute lymphoblastic leukemia in remission were exposed to microwaves by using a constant temperature waveguide irradiation chamber filled with 0.9% NaCl solution. All 3 specimens were exposed to 4 different power levels at 125, 250, 500 and 1000 mW/cm^2 for a duration of 15 minutes at 37°C, while sham exposure was conducted in the same chamber without power at 37° Celsius. The temperature of the chamber's solution during microwave irradiation showed a maximum increase less than 1°C at an incident power density of 500 mW/cm^2.

Both microwave and sham exposed cells were plated at an identical concentration of $4x10^4$ cells/plate in methylcellulose using fetal fibroblast conditioned medium as a stimulatory source. Colonies, a group of 20 or more cells were read on days 6&12 of culture. Comparison of colony number between sham and microwave exposed cell cultures showed a significant reduction ($P<0.01$) in cultures of cells exposed to 500 and 1000 mW/cm^2 power density, while cultures of cells exposed to 125 or 250 mW/cm^2 showed no reduction in colony number. All 3 specimens yielded similar results.

We conclude that 2450 MHz CW microwave may interact directly with human colony forming cells of the neutrophil cell lineage and that this interaction results in a CFC reduction as assayed by *in vitro* culture methods.

Human colony forming cells were exposed to 2450 MHz CW microwaves using a specially designed fluid filled waveguide exposure system (Lin and Peterson, 1977). The bathing solution, 0.9% sodium chloride, was kept at a constant temperature of 37°C by pumping it through a Lauda K-2/RD temperature bath. The solution temperature in the chamber during microwave irradiation showed a maximum increase less than 1°C at an incident power density of 500 mW/-cm^2 using a liquid crystal temperature sensor.

Human marrow cells were obtained from patients with acute lymphoblastic leukemia in remission. The aspirate (3-4 ml) was separated by mixing 1:1 with 3% dextran and letting it sit for approximately 1 hour. The buffy coat was removed and further separated on a ficoll-hypaque gradient, specific gravity 1.070, by centrifugation at 700 g for 30 minutes. The buoyant fraction was then washed, lysed using hypotonic treatment, and a non-adherent (NA) cell preparation was made by incubating the cells in α medium plus 20% FCS overnight at 37°C in a 100x15 mm glass petri dish. The following day NA cells were counted and suspended at a concentration of 4 to $8x10^6$ cells per ml in alpha medium plus 20% fetal calf serum. This cell suspension was used for both sham and microwave determinations as described below.

The cells were drawn into 20 µl glass micropipettes and placed one per exposure period in a sample holder which held them in constant position in the waveguide exposure chamber during microwave radiation. The microwave exposures were at incident power densitites of either 125, 250, 500 or 1,000 mW/cm^2 for 15 minutes. Other cells were sham irradiated by placing them in the sample holder with water bath flow on but without microwave radiation. Following microwave or sham treatment the cells were flushed from the micropipettes into 12x75 mm sterile tubes. A cell count and viability determination was then done on sham and microwave exposed cells.

Following the above treatment, the marrow cells were placed in a standard methylcellulose culture system at a concentration of $4x10^4$ marrow cells per plate. The stimulatory source was the supernatant from monolayer cultured fetal lung fibroblasts (FCM). Previously, we have shown that FCM stimulated human marrow CFC to form predominantly neutrophil colonies (Inoue and Ottenbreit, 1978). Cultures were placed in a 7.5% CO_2 incubator at 37°C, and neutrophil colonies, arbitrarily considered to be a group of 20 or more cells (Iscove et al. 1971), were scored on days 6&12.

The table (page 3) shows the number of colonies formed by marrow cells from the same sample after either sham exposure or microwave exposure at 4 different power levels. Each determination is based on a reading of quadruplicate plates $\pm$ on standard deviation.

Day of Culture	Sham	125 mW/cm^2	250 MW/cm^2	500 mW/cm^2	1,000 mW/cm^2
6	129±7	119±10	119±5	96±12	88±8
12	82±8	72± 8	75±2	59± 2	58±4

This experiment has been repeated 2 times with similar results. As can be seen from the culture data exposure at 500mW/-cm^2 and 1,000 mW/cm^2 caused a significant reduction in the number of colonies formed; probability $p < 0.01$ for determinations at days 6 or 12, while exposures at 125 mW/cm^2 and 250 mW/cm^2 did not cause a similar reduction.

Our results seem to indicate that 2450 MHz microwave irradiation may interact directly with human colony-forming cells of the neutrophil lineage and that this interaction results in a colony reduction as assayed by in vitro culture methods. However, further experimentation will be required before this concept can be fully evaluated.

REFERENCES

1. Inoue, S. and Ottenbreit, M.J. Blood 51:195-206, 1978.
2. Iscove, N.N., Senn, J.S., Till, J.E. and McCulloch, E.A. Blood 37:1-5, 1971.
3. Lin, J.C. and Peterson, W.D. Jr. Journal of Bioengineering 1:471-478, 1977.