AN EXPERIMENTAL MODEL FOR DETECTING AND AMPLIFYING SUBTLE RF FIELD-INDUCED CELL INJURIES.

[redacted]

A combination in vitro/in vivo experimental model utilizing neoplastic target cells, and a special receptive host, is capable of detecting subtle biological effects of non-ionizing, electromagnetic (EM) fields. The experimental findings carry provocative implications concerning the potential biological and pathological influences of radio-frequency (RF) and microwave irradiations, as well as suggesting possible useful applications for EM fields in experimental cancer therapy. Intrinsic, as opposed to hyperthermic, influences of 30 MHz fields upon cancer cells that were exposed in vitro, were detected only when such target cells were reimplanted subcutaneously (sc) in a receptive mouse strain specially selected for its immunocompetent equipoise. The effects of the RF fields on the neoplastic cells were detected by changes in the tumor latent periods, tumor incidence, tumor growth rates, maximum tumor volumes, tumor regressions, and survival times of the hosts. Hyperthermic effects associated with RF field exposure were controlled by: 1) utilization of the Guy cell-exposure apparatus and its sophisticated heat-exchange mechanisms, and 2) by the intentional induction of identical hyperthermias in sham-exposed and RF-exposed neoplastic cells in order to normalize the thermal factor. 3) The possible perturbing effects of thermal gradients were minimized by constantly circulating the cells through the sample chamber so that all cells were exposed to the same average thermal and RF environments. As a result of such controlled exposure of mouse lymphosarcoma cells to 30 MHz fields at 1 to 10 V/cm, a significant number of unexpected tumor regressions was observed. RF-exposed and sham-control neoplastic cells both produced tumors following sc implantation; however, a higher percentage of those tumors derived from cells that had been exposed in vitro to 30 MHz fields subsequently regressed, resulting in host survival. This was in contrast to the sham-exposed and control cells that produced a higher percentage of progressively growing lethal tumors. Mechanisms associated with this model appear to encompass a form of biological amplification that permits the reproducible detection of extremely subtle cell injuries resulting from specific RF field exposures.

These studies have so far been restricted to the single frequency of 30 MHz and electric field strengths of the order of 1, 5, and 10 Volts/cm. These particular field strengths yield average specific absorption rates (SAR) of 31 (±5), 352 (±68), and 1805 (±298) W/kg respectively.

SUMMARY

Tumor Incidence Following Neoplastic Cell Exposure to 30 MHz Fields in Vitro. Table 1 illustrates the striking difference that is observed in tumor incidence at 13 days following inoculation of receptive mice with neoplastic cells that had been exposed for 20 minutes at various field strengths within a hyperthermia-neutralizing environment of 42°C. It may be seen that while the zero-control preparation, which was also exposed to 42° for 20 minutes, produced tumors in 100 percent of the animals by day 13, no tumors had appeared where the neoplastic target cells had been exposed to RF field intensities of either 1, 5, or 10 V/cm

TABLE 1

Tumor incidence following irradiated neoplastic target cell implantation into C3H/Bi mice.

30 MHz [a] Irradiation (volts/cm)	Tumor Incidence [b] Day 13 Numbers	Day 13 Percent	Day 19 Numbers	Day 19 Percent	Statistical [c] Probability (t-test)
0	20/20	100	20/20	100	Reference
1	0/20	0	18/20	90	NS
5	0/20	0	19/20	95	NS
10	0/20	0	6/20	30	P < 0.0005

[a] Lymphosarcoma (6C3HED) ascites cells exposed to 30 MHz RF irradiation for 20 minutes in a hyperthermic neutralizing environment of 42°.

[b] Number of mice with tumors/Number of mice alive following irradiated neoplastic target cell inoculation.

[c] Student's t-test was employed in the calculation of the Day 19 probability values, using a total of 40 mice for each determination. The chi square test was also employed, yielding similar probability values.

of 30 MHz frequency.

At a later date in the experiment (day 19), tumors appeared in those animals inoculated with cancer cells that had been irradiated at 1, 5, or 10 V/cm. However, only 30 % tumor incidence occurred in mice receiving cells irradiated at 10 V/cm as compared with 100% incidence in the controls. Employing Student's t-test, these differences were highly significant statistically (P<0.0005). In this experiment there was also a significant difference in the tumor latent periods between the controls and the animals that had received tumor cells which had been exposed at 1, 5, or 10 V/cm.

Survival of Tumor-Bearing Mice Implanted with RF-Exposed Cells. Table 2 provides information on the survival time of the tumor-bearing mice as a consequence of the prior exposure of the neoplastic cells at various intensities of 30 MHz energy. The data show that 80 percent of the non-exposed control mice were dead by day 40 (20 percent survival),

TABLE 2

Accumulative, Tumor-Induced Deaths 40 days following neoplastic target cell implantation.

30 MHz [a] Irradiation (volts/cm)	Mouse Deaths [b] (Percent)	(No. Dead/No. of Mice)	Statistical [c] Probability (t-test)
0	80	16/20	Reference
1	15	3/20	$P < 0.0005$
5	25	5/20	$P < 0.005$
10	0	0/20	$P < 0.0005$

[a] Neoplastic target cells (6C3HED) were exposed to the RF power density indicated for 20 minutes in a hyperthermic neutralizing environment of 42°.

[b] Control and irradiated cancer cells were injected subcutaneously into 20 C3H/Bi female mice immediately following irradiation.

[c] P values were calculated using Student's t-test and employing 40 animals for each determination. The chi square test was also employed, yielding similar probability values.

in contrast to 100 percent survival of animals whose cancer cells were previously exposed at 10 V/cm. This implies that a cancer-cell modification occurs during or following 30 MHz irradiation. The beneficial effect for the host is also reflected by the latent periods, tumor growth rates, host survival time, and by the median time of mouse deaths, which were significantly longer in those animals that received cells irradiated at 5 and 10 V/cm as compared with the controls.

Tumor Regressions Following Non-Ionizing Radiation. The most interesting and perhaps the most important phenomena associated with RF irradiation of cancer cells are the delayed alterations in the tumor's behavior after exposure of the neoplastic cells to 30 MHz fields. See Table 3.

This Table illustrates the percentage of tumor regressions and non-takes among the various experimental groups. Only 10% tumor regressions were observed in sham-exposed control cells that received identical handling and instrument treatment at 0 V/cm. This is in striking contrast to the behavior of the tumors resulting from cells receiving 1, 5, and 10 V/cm. In this particular experiment, 70% tumor regressions occurred at 1 V/cm, and 90% tumor regressions at 10 V/cm.

TABLE 3

Percent tumor regressions and non-takes occurring in vivo following irradiation of neoplastic cells in vitro with various field intensities of 30 MHz RF continuous wave non-ionizing radiation.

30 MHz [a] Irradiation (volts/cm)	Combined Tumor [b] Regressions & Non-Takes (Percent)	Statistical [c] Probability (t-test)
0	10	Reference
1	70	$P < 0.0005$
5	50	$P < 0.01$
10	90	$P < 0.0005$

[a] Neoplastic target cells (6C3HED) were exposed to various field intensities of 30 MHz energy for 20 minutes in a controlled hyperthermic neutralizing environment of 42°.

[b] Target cells were inoculated subcutaneously into groups of 20 C3H/Bi female mice for each experimental condition. The values listed were obtained on experimental day 28 following target cell injection.

[c] Student's t-test was employed in the calculation of the probability values, using a total of 40 mice for each determination. The chi square test was also employed, yielding similar probability values.

The differences observed between 1 and 5 V/cm are not statistically significant from each other.

These studies have so far been restricted to the single frequency of 30 MHz and electric field strengths of the order of 1, 5, and 10 Volts/cm. These particular field strengths yield average specific absorption rates (SAR) of 31 (±5), 352 (±68), and 1805 (±298) W/kg respectively.