

THE COMBINED EFFECTS OF PULSED MAGNETIC RADIATION (DIAPULSE) AND CHEMOTHERAPY
ON TUMOR BEARING MICE.

THE MEASUREMENT OF RODENT PALATAL EXPLANTS AS A DEVICE FOR MEASUREMENT OF THE
BIOLOGIC EFFECTS OF NONIONIC RADIATION (EMR)

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SUMMARY

The aim of this program is to study the effect of nonthermal pulsed magnetic radiation on tumor growth and embryonic development.

Simultaneous treatment utilizing pulsed radiowave (diapulse) and cancer chemotherapy significantly extended the life span of BDF₁ mice with Lewis lung transplanted carcinoma. In comparison with nontreated controls, the combination of hydroxyurea and whole body nonionizing EM radiation (at 27.12 MHz) produced differential enhancement of longevity depending on power output. The highest power (38 mean watts) had the greatest effect. Hydroxyurea combined with highest power output achieved by pulsing the radiation 600 times per second; at a 3.9% duty cycle, peak watts = 975 produced the mean extension of life 67% greater than that of the group treated with hydroxyurea alone.

The stress involved in confining mice to holders and subjecting them to both nonionic radiation and chemotherapy produces significant variability in tumor growth. To achieve biologic quantification of EMR effects in vitro methods are necessary. We have studied palatal explants from 14 day old mouse embryos suspended in tissue culture as the EMR target. Histologic readouts of EMR effects can be obtained in 48 hours. This technique is difficult because of problems in timing mouse pregnancy to obtain properly dated embryos for quantitation of EMR effects of nonionic radiation on cellular differentiation, migration, adhesion, and destruction.

The effect of EMR on palatal explants and tumor growth can hopefully shed light on the significance of frequency windows vs. temperature effects, and provide significant information relevant to the use of nonionic radiation in the study of developmental birth defects as well as tumor growth.

INTRODUCTION

As discussed in our previous papers (refs. 1 and 2), the evidence supporting biological activity on nonionizing radiation based on either athermal or relatively low thermal mechanism needs serious evaluation. The diapulse instrument was studied because of its commercial availability and unverified reports of clinical anti-tumor activity. Diapulse has relatively low thermal effects as compared with diathermy despite similar reports of clinical usefulness in the treatment of injury.

This study was prompted by a report by Bassett et al., 1974, of inhibition of the transplanted METH-A sarcoma in mice by pulsed low energy electromagnetic fields (EMF). This led us to study the effects of pulsed magnetic radiation on tumor growth, normal development and drug metabolism in mice (refs. 1 and 2).

MATERIALS AND METHODS

The diapulse instrument operates at 27.12 MHz carrier frequency. Besides the commercial availability of this source of EMR (F.D.A. clearance is necessary), another immediate advantage was the existence of several field analysis studies and reports of its clinical use and biologic application (P. W. Neurath and J. Li, Personal Communication, 1974 and ref. 3).

Diapulse's theoretical design is based on the concept that pulsed high instantaneous power output (on the order of 280 to 975 watts peak), with relative low duty cycles (0.15% to 3.9%, depending on pulse repetition rate), should permit heat dissipation from tissues during the off-phase (1600 microsecond width or greater). In addition, the higher peak power levels of this instrument were designed to be capable, theoretically, of inducing tissue effects that could not be found with diathermy tolerance because of heat damage.

The diapulse generator used a fundamental frequency of 6.76 MHz, provided by a crystal, which is doubled and mixed with the output of a multi-vibrator stage. Power of the mixed stage is amplified, doubled and coupled to the 10 cm treatment head. Pulsed modulation as selected from 80 to 600 cycles per second is applied to the amplified stage to regulate the radiation supplied to the treatment head. As maximum settings, the treatment unit supplied 974 instantaneous watts with a 3.9% duty cycle, each pulse lasting 65 microseconds. This yields a highest average power output of 38 watts. These measurements were taken with a P80 probe placed on the inverted radiation head (1.3 cm removed from pancake coils) by means of 585A tektronic oscilloscope.

P. W. Neurath and J. Li (Influence of pulsed and continuous fields of a diapulse unit on peripheral circulation when applied to the abdominal region. Unpublished manuscript, personal communication with Medical Device Division, F.D.A., 1974), in a study to determine the magnetic and electrical field intensities of a diapulse instrument, reported similar measurements. A range of 10 to 45 volts/cm and an approximate 10 gauge field is seen at the surface of the head.

TUMOR STUDIES

Lewis lung carcinoma was obtained in the ascites form from serial passage in BDF₁ female mice and injected as a 10⁶ saline cell suspension subcutaneously into the flanks of mice of the same strain obtained from DBA paternal, C57 maternal parentage. Details of study are reported in our previous papers (refs. 1 and 2).

The purpose of this experiment was four-fold: (1) to determine the effect of a single drug, hydroxyurea (HU) or cytoxan (CTX), upon the tumor; (2) to determine the effect of a single drug plus irradiation 160-3 (approximately 4 mean watts) (low;lo) or 600-6 (38 mean watts) (high;hi) upon the tumor; (3) to determine the

effect of a combination of drugs (HU+CTX) upon the tumor; and (4) to determine the effect of a combination of drugs (HU+CTX) plus irradiation upon the tumor. The observed parameter was relative to change in lifespan of experimental animals over controls. Eight mice, matched as to age, sex, and weight, were treated in each group.

Chemotherapy

For more detailed analysis of methodology as relates to chemotherapy and dose timing of drug administration in relation to diapulse whole body irradiation, the reader is referred to our previous publication and our Airlie House Conference presentation (refs. 1 and 2).

Palatal Explants

The palatal processes are explanted following dissection from timed-pregnant Swiss Webster mice at day 14 of gestation in a 1:1 (V:V) mixture of Tyrode's hose serum. The explants are then positioned with the nasal surface resting on a millipore filter (0.3 μ m) porosity that is placed in contact with a nutrient agar-gel medium consisting of Hank's BME containing 1% agar, 1% dialyzed fetal calf serum, and 50 μ /ml penicillin-streptomycin. All cultures were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air and harvested at 24 or 48 hours following exposure to nonionic radiation. At harvest, explant cultures were fixed for electron microscopy and fusion was assessed according to the criterion of Smiley and Koch, 1971, and DePaola et al, 1974. (refs. 4 and 5). Mouse embryos are dissected at 14 days gestation and rabbit embryos at day 16.

Chemotherapy Results

There were two baseline controls. The first control group who were administered no drugs of any sort had an average lifespan of 36.86 days. The second group administered sodium pentobarbital to screen out effects of anesthesia on

experimental groups had an average lifespan of 37.8 days which was not significantly different.

Hydroxyurea Groups

The group receiving HU alone showed an average lifespan of 45 days with an increase of 22.1% over the controls at a significance level of 0.5. The group receiving HU plus irradiation at 160-3 (lo) (4 mean watts) showed an average lifespan of 38.75 days representing an increase of 5.1% over controls at a significance level of 0.75. The group receiving HU plus irradiation at 600-6 (hi) (38 mean watts) showed an average lifespan of 74.3 days representing an increase of 101.9% over controls at a significance level of 0.001.

Cytosin Groups

The group receiving CTX alone showed an average lifespan of 60.5 days representing a 64.1% increase over the controls at a significance of 0.05. The group receiving CTX plus irradiation at 600-6 showed an average lifespan of 64.17 days representing a 74.1% increase over controls at a significance level of 0.01. The group receiving CTX plus irradiation at 160-3 showed an average lifespan of 76.14 days representing a 106.6% increase over controls at a significance level of 0.005.

Multiple Drug and Irradiation Groups

The group receiving a combination of HU and CTX showed an average lifespan of 21.67 days representing a 41.2% toxic decrease in lifespan compared with non-chemotherapy controls. The group receiving a combination of HU and CTX plus irradiation at 160-3 showed an average lifespan of 50.86 days representing a 38% increase over nonchemotherapy controls and a significance level of 0.01 from the HU and CTX controls. The group receiving a combination of HU and CTX plus irradiation at 600-6 showed an average lifespan of 37.5 days which is an increase of only 1.7% over nonchemotherapy controls.

The groups in order of decreasing importance (experimental groups compared with control groups): combination CTX and irradiation 160-3 showing increased lifespan (I.L.S.) of 106.6%; combination HU and irradiation 600-6 showing I.L.S. of 101.9%; combination of CTX and irradiation 600-6 showing I.L.S. of 74.1%; CTX alone showing I.L.S. of 64.1%; combination of HU, CTX, and irradiation 160-3 showing I.L.S. of 38%; and HU alone showing I.L.S. of 22.1% over controls. The combination of HU and CTX without irradiation showed a decrease in lifespan of 41.2%.

Similar studies with bleomycin given at these same radiation power levels and pulse frequencies were not significantly different from the nonchemotherapy controls or the bleomycin alone controls.

In no case was weight loss a factor, nor did pentobarbital by itself exert any therapeutic influence on the results seen. There was no evidence of body temperature elevation on assessing rectal and subcutaneous temperature by thermistor following power termination in a matched BDF₁ mice group.

Palatal Studies

This program is in progress and unfortunately technical problems related to timing of mouse pregnancy and palatal harvest suggest at this time that as a biological assay of nonionic magnetic radiation, this method of biologic assay has variables equal to that of tumor growth studies in the living mouse.

DISCUSSION

In our previous study (ref. 1) in unanesthetized mice, growth of Lewis lung carcinoma tumors were either enhanced or inhibited depending on the scheduling of the EMR exposure used. Pre-tumor inoculate irradiation, one hour daily for one week, resulted in a 49% inhibition in mass at post-tumor inoculate day 14 and 45% inhibition at day 26 after exposure to a 6 mean watt (160 pps/585 watts) power level. However, extended scheduling using daily irradiation for 13 pre-and 6 post tumor inoculate days, at the same power level, resulted in a contrasting 20% stimulation in tumor growth and the use of higher power levels was associated with greater increased growth of tumors of 54% and 51% (15 mean watts produced by 400 pps/585 watts and 38 mean watts by 600 pps/975 watts, respectively, given for 13 pre-and 14 post-tumor inoculation days). In contrast, anesthetized mice receiving identical treatment (160 pps/585 watts daily seven pre-and six post tumor inoculate days) demonstrated a greater inhibition of tumor mass, 67%, in comparison with the unanesthetized animals, 20%. This may indicate an effect of temperature elevation in view of our data demonstrating heat retention in irradiated anesthetized mice (ref. 1).

Simultaneous treatment utilizing pulsed radiowave (Diapulse) and cancer chemotherapy significantly extended the life span of BDF₁ mice with Lewis lung transplanted carcinoma. In comparison with nontreated controls, the combination of hydroxyurea and whole body nonionizing EM radiation (at 27.12 MHz) produced differential enhancement of longevity depending on power output. The highest power (38 mean watts) had the greatest effect. Hydroxyurea combined with highest power output achieved by pulsing the radiation 600 times per second; at a 3.9% duty cycle, peak watts of 975

produced the mean extension of life 67% greater than that of the group treated with hydroxyurea alone.

The stress involved in confining mice to holders and subjecting them to both nonionic radiation and chemotherapy produces significant variability in tumor growth. To achieve biologic quantification of EMR effects, in vitro methods are necessary. We are now testing palatal explants from 14 to 16 day old mouse or rabbit embryos suspended in tissue culture as the EMR target. Histologic readouts of EMR effects can be obtained in 48 hours. This technique might enable us to quantitate EMR effects of nonionic radiation on cellular differentiation, migration, adhesion, and destruction. The effect of EMR on palatal explants can shed light on the significance of frequency windows against temperature effects and can provide significant information relevant to the use of nonionic radiation in the study of development birth defects as well as tumor growth.

The importance of nonionic low thermal radiation is seen in the recent IV International Bioelectrophysiology Meeting at Woods Hole. Low energy nonionic radiation effects have been seen to stimulate bone repair (refs. 6 and 7 and C.A.L. Bassett, personal communication, 1977), derepress frog embryo cells and convert them to fibroblasts (ref. 8), and produces dendritic outpouchings in cultured neuronal cells (ref. 9) and control dorsal ectoderm migration in spinal cord development (ref. 10). The energy levels for these effects are infinitesimal and unrelated to heat production and evidence by references 11 and 12 and others (ref. 8, 10 and 13) support that this condition is associated with calcium flux (refs. 14 to 16) and may require frequency windows (specific frequencies) for selected effects to be seen. Calcium ionophores (ref. 8, 10, and 13) can mimic at

least some of the effects reported for low energy electromagnetic fields on cell physiology.

In view of frequency window data indicating that low energy pulsed EMF can affect a variety of biologic systems with stimulation of bone repair and inhibition of tumor growth, further work remains to be done in testing varied frequencies for optimal effect. This is important in view of Holt's (ref. 17) report of increased survival of head and neck cancer where microwave at 434 MHz combined with radiotherapy was reported to increase 2 year survival of recurrent head and neck epidermoid cancer. Holt insists that the frequency of 434 MHz is critical for results seen.

Our future work will explore frequency window effects ranging from 8 to 400 MHz. With new instrumentation from Bassett's group and NASA, we will avoid the high energies that produce heat effects although, as was seen in the diapulse system using a pulsed magnetic field, minimal heat was generated in both the in vivo mouse tumor system and in tissue culture palatal explants.

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Questions and Answers Following Regelson's Paper

Beebe: Did you consider the effect of the diurnal cycle on the susceptibility?

Regelson: Well, that is obviously a factor, but all our work was done during the day.

Beebe: Was it done routinely in the morning?

Regelson: Yes. Routinely, our timing was fixed so that as a variable we did not change it. Once we locked in, we were locked in. Incidentally, the light cycle in the mouse room was controlled.