A SYSTEM FOR DEVELOPING MICROWAVE INDUCED HYPERTHERMIA IN SMALL ANIMALS

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Abstract

A system for developing microwave induced hyperthermia in small animals is described, for the purpose of assessing the effects of heat alone and heat plus ionizing radiation on normal tissue and tumors. The temperature is monitored using thermistors, and with the use of a scanner, recorded on a single channel recorder. The thermometry technique is still under review. Thermographic studies on a sacrificed animal and thermistor measurements in a phantom reveal a pattern of deep heating in a well defined region. Preliminary biological measurements are presented, but currently long term animal survival remains a problem.

Introduction

The earliest recorded attempt to use heat to cure tumors is found in the Edwin Smith Papyrus[1] which is believed to have been written about 3000 B.C. This involved the use of a so-called fire drill which was a smoldering stick of wood inserted into the tumor. More modern methods, i.e., the use of electrostatically generated heat, were suggested soon after Hertz[2] in 1886 first produced oscillating currents in a sustained manner. Within a few years d'Arsonval, a physician-physiologist in France suggested their use in medicine. deKeating-Hart applied a modified d'Arsonval circuit capable of producing a spark to destroy tumor tissue and demonstrated the procedure at the International Congress of Electrology at Milan in 1906. In the early 1900's what we would now call long wave diathermy was used to treat a variety of conditions. In about 1928 short wave diathermy came into being and many exhorbitant claims were made concerning therapeutic benefits for a variety of conditions with numerous machines that were then being marketed. Denier[3] in France (1939) used microwaves combined with x-rays against tumors (80 cm wave length). By this time (1937) more than 750 articles and 18 books had been devoted to short wave diathermy. Following the intense technological development of microwave generating devices during the second World War, numerous reports[4,5] on the ability of microwaves to heat living tissue appeared in the literature. Consierable activity in this area has been carried out more recently, in the 1960's and 70's in this country. The several reports of Crile[6,7] predate the more recent quantitative in vitro cell culture studies, but these articles are intriguing for their provocative insights. Crile reported on systematic studies involving tumors transplanted to the foot pad of mice, spontaneous tumors in dogs and in patients, and determined some empirical dose-time relationships for the heat treatment. He observed that if the tumor cells of the transplant are previously heated in vitro, or heated in vivo and then transplanted to another animal, the tumor grows as if it had not been heated at all, even though subjected to a normally lethal dose of heat (determined empirically in vivo).

In 1971, Westra and Dewey[8] published a systematic, quantitative study of in vitro cell survival curves. It is not appropriate to review this type of data thoroughly here, but some general features of the results will be mentioned. When the logarithm of the surviving fraction is plotted against time of treatment, a family of curves (temperature dependent) is obtained, with higher temperatures producing a steeper slope. These curves are qualitatively similar to ionizing radiation cell survival curves, with a shoulder, an exponential portion, and demonstrate the cells capacity for sublethal repair, as the shoulder re-appears in a fractionated treatment. From their data they calculate an activation energy of 141 kcal/mole, consistent with heat denaturation of several proteins and enzymes, which they postulated. (Activation energies for heat induced damage to DNA are much lower, of the order of 25 kcal/mole). These studies involved an asynchronous population. For synchronous cells, they found a cell cycle dependence essentially the reverse of that for x-irradiation. Palzer and Heidelberger[9] reported a 7 fold dependence upon cell cycle phase (HeLa cells), and that hyperthermia inhibited various processes of macromolecular synthesis.

In vivo studies, particularly those involving the effects of heat on normal tissues, are less numerous. Robinson[10], in studies involving the enhancement effect of heat on ionizing radiation, reported the provocative result that tumors (C3H mouse mammary) were characterized by greater TER's (thermal enhancement ratio) than normal mouse skin, thus producing a favorable therapeutic ratio. In this report, the ratio of the TER for the tumor to that of normal mouse skin, (therapeutic ratio), increased with increasing temperature to a value of 2.10 at 43.0°C.

Zimmer[11] reported the selective heating of tumors in small laboratory animals, and although the goal (drug uptake by a tumor in a hypothermic animal) was different than ours, it is a very interesting technique in that he was able to maintain a 25°C differential between the tumor and whole body temperature (body in deep hypothermia and tumor at normal temperature). Mendecki[12] has reported dramatic cure rates with all treated animals (mammary adenocarcinoma implanted in C3H mice) cured after two applications of microwave induced local hyperthermia, without any other effects from the heat, while all non-treated controls died. Marmour, Hahn and Hahn[13] reported similarly dramatic cures for the EMT-6 sarcoma tumor in mice and only somewhat more resistance to cure for the KHJJ carcinoma in Balb/Cka mice. They used radio frequency heating (13.56 MHz) in vivo, and in an effort to explain the remarkable effectiveness of the techniques, performed tumor cell survival studies, and concluded that direct cell killing by the RF heating alone could not explain the dramatic cure results. One possible mechanism suggested by them involves host immune response, and they point out that this postulate is consistent with the relatively higher thermal resistance to cure of the KHJJ, which is less immunogenic than the EMT-6. Thus some of the much earlier results and suggestions of Crile are now being confirmed.

Robinson[14] describes a system for heating an extruded, transplanted tumor using 2450 MHz, which he describes as marginally effective (excessive temperature gradient in tumor), and consequently the free field microwave heating was augmented using warm air. The tumors were 1 cm in diameter, and this result illustrates the inherent problem of depositing the microwave energy deeply in tissue.

The apparatus described in this report was designed to achieve deep heating, e.g. in the abdomen of a mouse, to assess thermal effects on normal tissue and a variety of tumors. It appears that the immune response may play a pivotal role, and it was desired to be able to assess effects at various sites without being restricted to extruded and transplanted tumors.

Method

For deep, non-invasive heating of living organisms, ultrasonic and electromagnetic techniques suggest themselves. The modality of heating using microwaves

was chosen for this study, primarily because
a) this modality will be employed extensively in clinical trials (currently underway) and the expertise acquired will be valuable.
b) there is a considerably more extensive body of literature concerning this technology, as compared to ultrasonic heating of living organisms.

Table I gives an abbreviated list of conventional rectangular waveguides available, and a glance at this table reveals an inherent dilemma: to choose a frequency providing good depth of penetration, results in too large an applicator, if it is to be air filled.

Thus numerous reports in the literature, e.g. Guy[15,16] employ dielectric loading, allowing the designer greater range of applicator sizes, while still achieving good depth of penetration. A system using this principle, while attempting to provide maximum flexibility in the timing of microwave heating and x-irradiation, has been described elsewhere.[18]

For our specific biological application it was felt that the end points in the first phase of the experiments had been chosen such that it was desirable to heat an entire transverse section of the experimental animal (mouse). Difficulties were envisioned with the contact applicator approach, e.g. lack of reproducibility due to variations in positioning, curvature, air gaps, etc. These considerations gave rise to the applicator described in this report.

Typically an investigator chooses an ISM frequency, e.g. 915 MHz or 2450 MHz, and then attempts to optimize an applicator for that frequency. In these animal studies an alternate approach was chosen. That is, a general purpose laboratory quality radio frequency power supply with broad frequency range capability was selected, and then an applicator was designed to achieve the desired goal, i.e. that of heating the entire transverse section of a mouse. Then the applicator need not be optimized for that frequency but rather the generator can simply be tuned by the turn of a dial to achieve optimum results with that applicator.

The unit selected to develop the microwave power is a model 15022 RF Power Supply (MCL, Inc., La Grange, Ill.), which can excite anyone of six available planar-triode cavity oscillator plug-in units, covering a total frequency range from 10 MHz to 2500 MHz. Power available depends upon the frequency, as is shown in Figure 1, but is adequate at 915 MHz for human patient applications. Two such cavity oscillator plug in units are on hand, the model 6050 and model 6051, providing a continuous frequency range from 500 MHz to 2000 MHz, at the turn of a dial.

The basic philosophy underlying the design of our applicator can be simply stated. In the TE_{10} mode, the "a" dimension (width) determines the cut-off frequency, and the "b" dimension (height) is irrelevant. Thus a relatively large waveguide (WR-650) is used, to allow use of a low frequency to insure good depth of penetration, but of reduced height to limit the region of heating. The mouse, in a plastic test tube, is inserted in a 1" dia. hole centered in the broad face of the waveguide, which due to its small diameter is non-radiating. (See Figure 2).

To test the viability of this approach, a prototype (using WR-430 components) of the system shown in figure 2 was constructed. Thermographic studies were performed on a sectioned mouse (cleaved along the saggital plane, after freezing in liquid nitrogen). These two halves were then reunited, and heated (from room temperature) in the applicator. A clinical thermographic camera was previously set up by focussing on a beaker of water at 43°C in a 23°C ambient background. The mouse was removed, situated in place of the beaker, and photographed, as rapidly as possible to preclude substantial thermal diffusion. These thermograms consistently revealed not only deep heating, but preferential heating in the center of the animal, and qualitatively, a reasonable degree of symmetry in the heat deposition pattern. Subsequently custom components were fabricated compatible with a WR-650 coax-to-waveguide adapter.

Thermometry and Recording System

Thermistors encapsulated in a teflon sheath(0.024") were chosen for the temperature monitoring (α = 4%/°C). Unfortunately, in this small diameter clinical series of probes, the thermistors are not specified as being interchangeable, which presents a problem when using an array of thermistors in a scanning mode, such as in our application. The signals from the thermistor array are scanned by the Keithley low voltage scanner (see Figure 3), and recorded on the Heath single channel, 1 volt recorder via the Heath 18 range potentiometric amplifier (capable of displaying signals from 1 mV to 500V full scale on the recorder). An associated module is the dc offset module which provides for scale and range expansion.

Applicator and Results

An applicator was fabricated and is shown in Figure 2. It consists of a conventional WR-650 coax-to-waveguide adapter, followed by the electrically compensated transition section (tapered in the "b" dimension only) to adapt the larger component to the reduced height waveguide test section. The remaining waveguide component is a reduced height coax-to-waveguide adapter, enabling the power to be dissipated in the dummy load. Three test sections are on hand, with the "b" dimensions 1 1/4", 1" and 3/4". These may be adapted to the two contiguous components by quarter wave transformer sections. Measurements indicate that with the test port empty, >99% of the power delivered to the WR-650 component is delivered to the dummy load. With a mouse in the test port, it is found that about 20% to 50% of the power incident upon the mouse is reflected back to the generator, up to about 20% of the incident power reaches the dummy load (dependent upon the choice of many parameters, such as frequency, etc.) with the remainder being absorbed by the mouse.

To test how effectively the system limits the microwave heating to the region defined by the interior of the waveguide, the data shown in Figure 4 was obtained, using thermistors in the center of the waveguide, and in the center of the "guard rings". Figure 5 shows a preliminary experimental run with the three thermistors placed as indicated in Agar and NaCl phantom. (This simplified phantom material will not adequately simulate the loss tangent of tissue, but it was felt would be suitable to gain an idea of the relative heat distribution pattern. These results are still under investigation, but appear qualitatively similar to those obtained using thermographic techniques in a sacrificed animal which showed preferential heating in the center). To test the validity of thermistor readings in the microwave field, camparisons of the thermistor with an alcohol thermometer, both in the phantom in the interior of the waveguide were made.

At low power levels, e.g. 4 or 5 Watts, which is the level required to maintain a constant temperature of ~ 43°C in a phantom, the thermistor appears to read ~0.25°C high, which can also be demonstrated simply by turning the power off. The interaction of one thermistor with another in the field is not precluded however.

The frequency of 1210 MHz is somewhat arbitrary, but was chosen on the basis of measurements that indicated maximum energy absorption in the test animal at this frequency. Higher frequencies result in altered thermal distribution patterns (less symmetrical, with higher T nearer generator). For use at 915 MHz, larger components would be required.

Biological results to date are still preliminary, and the techniques are currently under review. In an initial trial, 7 mice were anesthetized with 70-90

mg/kgm of Diabutal (a barbiturate anesthetic) and heated according to either of two regimens; 40°C for 25 minutes or 43°C for 15 minutes. (Temperature was monitored as indicated in Figure 6 (i.e., one thermistor centrally located in the microwave field, and one thermistor outside the field to monitor whole body temperature). Long term animal survival was not obtained. One LAF$_1$ mouse died 16 hours post-heating to 43°C in the abdominal region, while the remaining mice (Balb/C) survived lesser times.

In a second trial experiment, the anesthetic dose was reduced to 60 mg/kgm Diabutal and two thermistors in different anatomical positions, were used to monitor the temperature in the microwave field. One thermistor was inserted 1 inch into the rectum (final position being mid-abdomen), while the second thermistor was implanted subcutaneously using a trocar (subsequently withdrawn), leaving the thermistor in place at mid-height in the waveguide, as was the rectal thermistor. Rotation of the test tube containing the mouse (in an attempt to obtain more uniform heating), resulted in the observation that the subcutaneous thermistor reading was essentially independent of angular orientation (near microwave generator or near load), and consistently $\sim$1° to 2°C warmer than the rectal thermistor. 5 out of 5 Balb/C mice heated to 40°C as indicated by the subcutaneous thermistor (15-45 minutes) are alive and appear healthy 5 days post-heating.

References

1. The Edwin Smith Surgical Papers, Chicago, 1930. (Cited in Licht, Ch.VI, History of Therapeutic Heat, in Therapeutic Heat and Cold, S. Licht (ed), E. Licht (publ.), 1965.
2. Hertz, H. Electric Waves, London, 1900.
3. Denier, A. Les ondes hertziennes ultracourtes de 80 cm. J. Radiol. Electrol., 20:193, 1936.
4. Krusen, F. H. Medical Applications of Microwave Diathermy. Proc. Roy. Soc. Med., 43:641, 1950.
5. Osborne, S. L. and Frederick, J. N. Microwave Radiation. J.A.M.A., 137:1036, 1948.
6. Crile, George, Jr. Selective Destruction of Cancers after Exposure to Heat, Ann. Surg. 156: 404, 1962.
7. Crile, George, Jr. The Effects of Heat and Radiation on Cancers Implanted on the Feet of Mice, Cancer Research 23: 372:380, 1963.
8. Westra, A. and Dewey, W. C., Variation in Sensitivity to Heat Shock During the Cell Cycle of Chinese Hamster Cells in Vitro, Int. J. Rad. Biol., Vol. 19, No. 5, 467-477, 1971.
9. Palzer, R. J, and Heidelberger, C. Influence of Drugs and Synchrony on the Hyperthermic Killing of HeLa Cells, Cancer Res. 33, 422-427, Feb. 1973.
10. Robinson, J. E., Wizenberg, M. J., McCready, W. A. Radiation and Hyperthermal Response of Normal Tissue in Situ, Rad. Biol. 113, 195, Oct. 1974.
11. Zimmer, R. P., Ecker, H. A., and Vopavick, V.P., Selective Electromagnetic Heating of Tumors of Animals in Deep Hyperthermia, IEEE Trans. Vol. MTT-19, Feb. 1971.
12. Mendecki, J., Friedenthal, E., and Botstein, C., Effects of Microwave-induced Local Hyperthermia on Mammary Adenocarcinoma in C3H Mice, Canc. Res. 36, 2113-2114, June, 1976.
13. Marmour, J., Hahn, N., and Hahn, G. M., Tumor Cure and Cell Survival after Localized Radiofrequency Heating, Cancer Res. 37, 879-883, Mar. 1977.
14. Robinson, J. E., McCulloch, D., Edelsack, E. A., Microwave Heating of Malignant Mouse Tumors and Tissue Equivalent Phantom Systems, J. Micro. Pow. 11 (2), 87, 1976.
15. Guy, A. W. Electromagnetic Fields and Relative Heating Patterns Due to a Rectangular Aperture Source in Direct Contact with Bilayered Biological Tissue, IEEE Trans Micro. Theory and Tech., Vol. MTT-19, No. 2, Feb. 1971.
16. Lehman, J. F., Guy, A. W., et al, Evaluation of a Microwave Contact Applicator, Arch. of Phys. Med. and Rehab. 143, Mar. 1970.
17. Johnson, C. C., Guy, A. W., Nonionizing Electromagnetic Wave Effects in Biological Materials and Systems, Proc. IEEE, Vol. 60, No. 6, 692, June 1972.
18. Baker, R. J. and Smith, V., Experimental Arrangement for In Vivo X-Ray and Microwave Irradiation of Small Animals, 4th Int. Conf. on Med. Phys., Spec. Issue of Phys. in Can., Vol. 32, 35.8, July, 1976.

TABLE I

EIA Designation	Dimensions a(cm)	Dimensions b(cm)	Recommended Operating Range (GHz)	Cut off frequency (GHz)	Cut off wavelength (cm in air)	Depth of Penetration at λ_c (cm)[a]
WR-650	16.51	8.255	1.12 - 1.70	0.908	33.0	3.05
WR-430	10.92	5.461	1.70 - 2.60	1.375	21.8	2.55
WR-340	8.636	4.318	2.20 - 3.30	1.686	17.3	2.30
WR-137	3.48	1.58	5.85 - 8.20	4.301	7.0	1.09
WR-112	2.85	1.26	7.05 - 10.0	5.260	5.7	0.61

[a]Defined as depth at which E falls to a value of 1/e, or power density decreases by a factor e^{-2}. Values interpolated from Table I of reference 17, and pertain to a plane wave irradiation of a homogeneous, planar muscle equivalent phantom or tissue in the absence of ground plane effects.

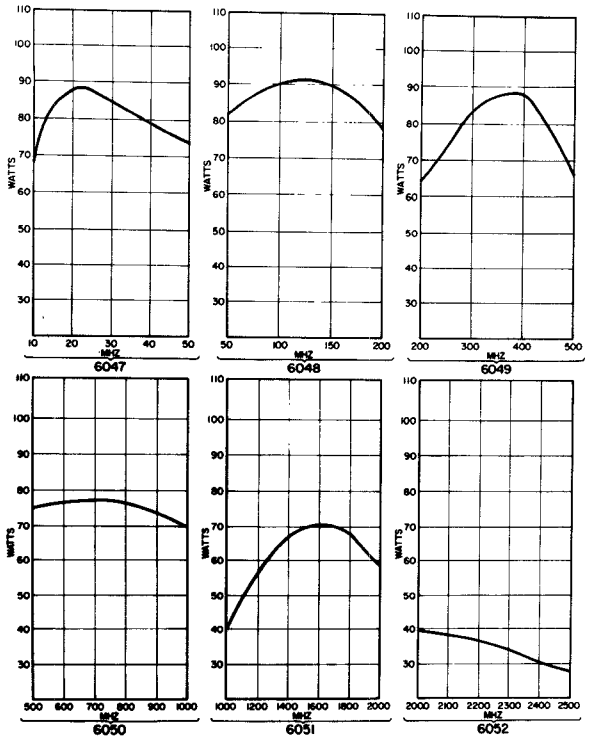

Figure 1. Specifications of MCL model 15022 RF power generator with plug-in units.

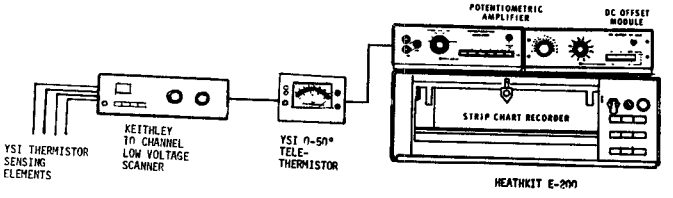

Figure 3. Thermometry and recording system, consisting of thermistor array, scanner, meter, and recording components.

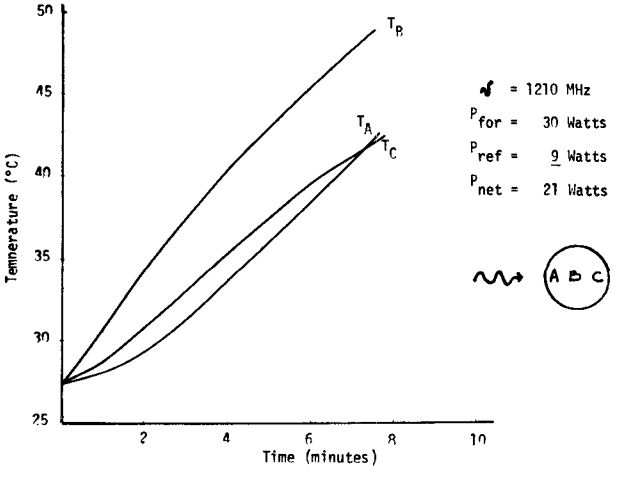

Figure 5. Thermistor measurements at position A,B,C, showing slightly preferential heating rate at the center of a simulated mouse phantom.

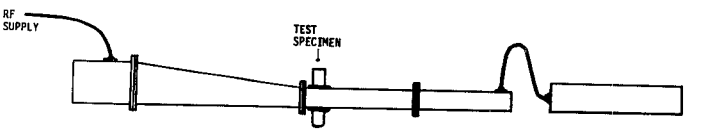

Figure 2. Lateral view of microwave applicator system. Waveguide components, left to right, are a conventional WR-650 coax-to-waveguide adapter, an electrically compensated one dimensional transition section, tapering to a reduced height test section, a reduced height coax-to-waveguide adapter, followed by a coaxial dummy load.

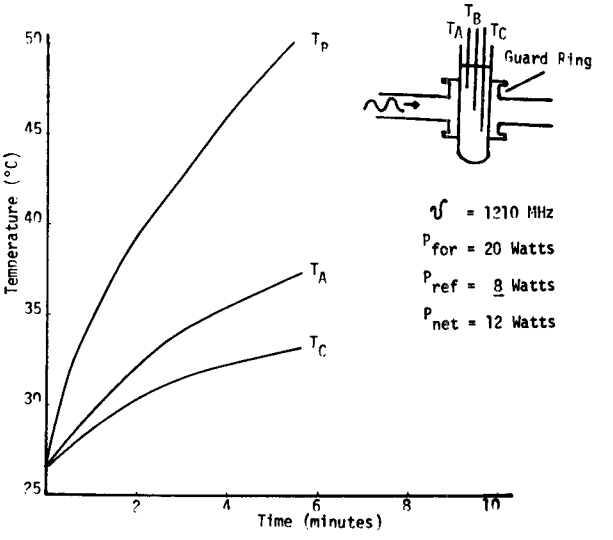

Figure 4. Heating developed in thermistors located 0.6 cm outside of the waveguide (T_A, T_C) compared to thermistor located at center of waveguide (T_B).

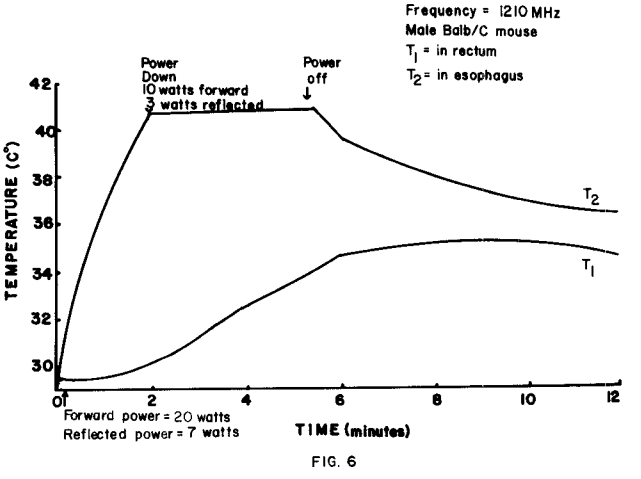

FIG. 6

Figure 6. Thermistor measurements in a live mouse, with T_1 located in the rectum (outside of the microwave field, monitoring whole body temperature) and T_2 in the esophagus (lungs exposed to the microwave field).