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In Vivo Spectroscopic and Imaging Studies of Photosensitizers in Photodynamic Therapy

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Abstract

Photodynamic Therapy (PDT) has emerged as a useful cancer treatment modality which utilizes a tumor localizing dye and activating light to selectively destroy neoplastic tissue. In an effort to understand the newly synthesized photosensitizers, we are studying them in a mouse tumor model grown on the dorsal side of the foot by *in vivo* magnetic resonance techniques. We have synthesized several photosensitizers which are specifically labeled with fluorine. Several coils appropriate for the tumor study by ^{19}F NMR were designed and constructed for this project. The solenoid coil tunable to both ^{1}H and ^{19}F nuclei was used to monitor the ^{19}F labeled photosensitizer in the mouse foot tumor. An *in vivo* ^{19}F NMR technique was used to study the retention of the photosensitizer over time in the tumor. We have used ^{31}P NMR to study the outcome of PDT after using the new photosensitizer.

Introduction

Magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI) have significant impact on both basic research and clinical applications due to their non-invasive nature and sensitivity to molecular structure, interactions, and mobility. Additionally, MRS can be quantitative under proper conditions and MRI can provide morphological images.

Photodynamic Therapy (PDT) is an expermimental cancer treatment modality which selectively destroys cancer cells by interaction of light with a photosensitizing dye, presumably via singlet oxygen formation (Weishaupt et al., 1976). There are, however, some questions which need answers, such as 1) the time period for which photosensitizers are retained in the tumor, and 2) the optimum time at which laser irradiation can ve initiated. A non-invasive way of monitoring the photosensitizer in the tumor would be useful in PDT studies and magnetic resonance is one such useful tool.

Extensive research on photosensitizer localization in cancerous tissue has been reported (Kessel and Chou, 1983; Dougherty et al., 1984; Swincer et al., 1984). Researchers have detected the presence of photosensitizers in cells (Bottiroli et al., 1984; Moan, 1984) using their fluorescence properties. The measurements of absolute concentration of the photosensitizers *in vivo* have been attempted and have been found to be extrememly difficult because of the dependence of fluorescence efficiency on the tumor tissue, and due to the fact that the fraction of detected emitted photons depends upon the tissue type, the source, and the detector geometry.

Monitoring the effect of PDT through *in vivo* ^{31}P MRS has been used in biomedical *in vivo* sutdies, and this method has revealed alterations in energy and phospholipid metabolism before and after laser irradiation. Ceckler et al. (1986) have reported dramatic and often near complete decreases in nucleoside triphosphate (NTP) peaks accompanied by significant increase in inorganic phosphate (Pi) within four hours of PDT treatment. Completely non-invasive MR can provide a measure of the concentration of the photosensitizers and their metabolites within tumors. Detailing pharmacokinetics and drug concentrations within the tumor will help determine the minimum doses to insure the fewest side effects for humans undergoing PDT. These studies will aid in the development of a more effective protocol for humans.

In this paper we will discuss: 1) the design and fabrication of RF coils for MRS and MRI tumor studies, 2) tumor bioenergetics monitored by ^{31}P MRS, and 3) the detection of the ^{19}F labeled photosensitizers in radiation induced fibrosarcoma (RIF) tumors *in vivo*. We chose fluorine as the labeled element because the ^{19}F isotope has 100% natural abundance, a spin of 1/2, and an NMR sensitivity that

is 83% that of hydrogen. *In vivo* studies of porphyrin photosensitizers are very limited. In fact, to our knowledge, there are no known reports of *in vivo* MR studies attempting to detect such sensitizers in cancerous or non-cancerous tissue. Comprehensive knowledge of the extent of localization and the rate of accumulation is of immense value in PDT.

Materials and Methods

MRS and MRI Coils.--Figure 1A shows an air core solenoid coil with four equally spaced turns which is 1.5 cm in diameter and 2.1 cm in length. In order to tune to the resonance frequencies for both ^{1}H and ^{19}F and to insure a good circuit quality factor, Q, we mounted the copper wire on a Plexiglas (Rohm and Haas, Canada, Inc.) insulator. The fixed capacitors were 2.5 pF (Dielectric Laboratories, Inc.) In order to maximize the tuning range, we had to minimize the scattering capacitance and any connector resistance. Frequency properties were measured with a Wiltron 6400 Series RF Network Analyzer.

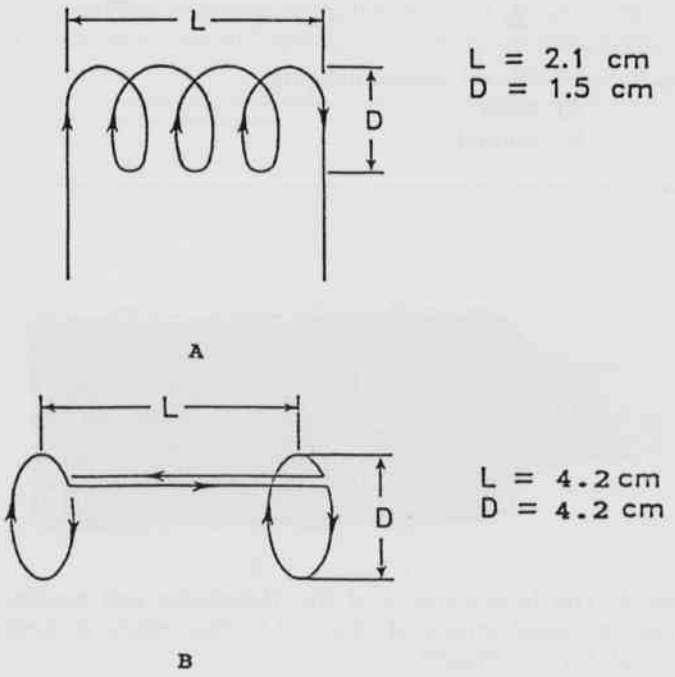

Fig. 1A. The dimensions of the solenoid coil
1B. The dimensions of the Helmholtz coil

Helmholtz Coil.--To insure a uniform imaging region for the tumor studies, the diameter and the length of the coil were optimally designed. The diameter, d, was chosen approximately equal to the length, a, (d = 4.2 cm; a = 4.2 cm), to maximize the homogeneity of the field region. Fig. 1B shows the geometric design of the Helmholtz coil. The coil was constructed by first winding a 3.30 mm wide by 0.08 mm thick adhesive backed copper foil on the cylindrical fluorine free tubing. The coil was glued to a Plexiglas plate to avoid any movement which adversely would effect the resonant frequency. All connections were short to minimize the resistance and scattering capacitance. Also, we used fluorine free capacitors and materials.

PDT on the RIF Tumor.--Fresh RIF cells were injected into the flank of male mice (C3H/HeN). After an appropriate tumor size was reached, it was cut open and a small piece of the tumor was implanted on a mouse foot. A mouse foot tumor of proper size (400-600 mm^3 for imaging and spectroscopy) was obtained after 10 to 15 days.

Several background control spectra were collected with only the coil and the coil with a typical tumor. No fluorine peaks were observed. The ^{19}F labeled photosensitizer compound was dissolved in distilled water and the spectrum was recorded in the GE 4.7T animal imager using the solenoid coil in the balanced configuration. The photosensitizer was injected IP (25 mg/kg) and after 24 hours PDT treatment was initiated wtih a laser power of 150 mW/cm^2. The total light energy was 50-100 joules at the photosensitizer absorption wavelength of 630 nm. The Ar+ CW pumped dye laser output was coupled via an optical fiber in the PDT experiment. All ^{31}P spectra were obtained on a General Electric 4.7T animal imaging system. A home-built phosphorus coil was used in acquiring spectra. Labeled ^{19}F photosensitizer was detected *in vivo* by using the home-built balanced solenoid coil.

Results and Discussion

Comparison of the Balanced and Unbalanced Coils.--The coil configuration is a very important factor in determining the frequency response of both the solenoid coil and the Helmholtz coil. Both the balanced and unbalanced configurations of the solenoid coil (as shown in Figs. 2A, 2B) were tested. The balanced coil was found to be more symmetrical about its resonant frequency compared to the unbalanced coil.

Coil Performance Tests Using Phantom.--The purpose of building coils tunable to both ^{1}H and ^{19}F nuclei was to perform proton imaging, as well as fluorine spectroscopy or imaging, using the same coil without disturbing the animal. In addition it also helps in shimming the region of

interest using the proton frequency since all biological samples have large amounts of water, whereas, the amount of naturally occurring fluorine compounds are so small, they give no background signal. Furthermore, to obtain fluorine signals from a localized region of interest, we need proton images as a reference or guide.

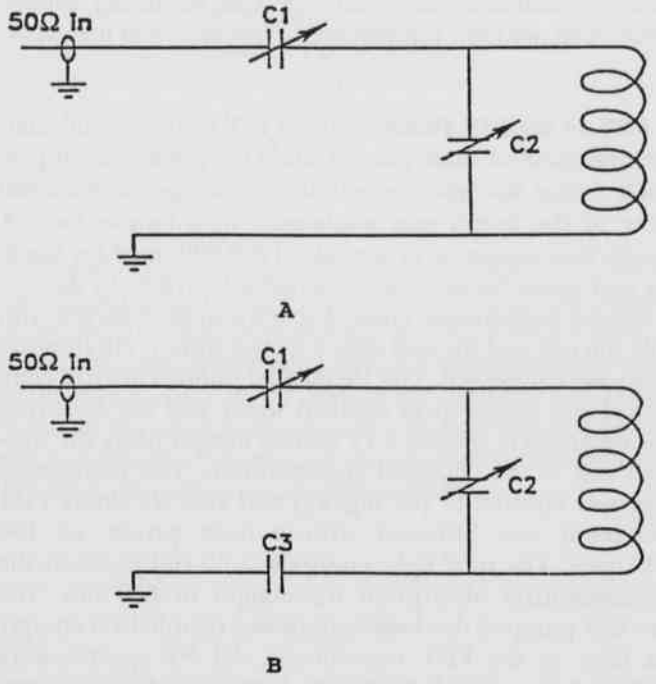

Fig. 2A. Schematic of the unbalanced coil
2 B. Schematic of the balanced coil

A phantom is an ideal sample for testing the imaging and spectroscopic properties of the doubly-tuned coil. Fig.3 shows the phantom images for the balanced solenoid coil in both the axial and sagittal directions and Fig. 4 shows the sagittal image from the Helmholtz coil. From these images, we concluded that the solenoid coil was not suitable for the imaging studies. The Helmholtz coil has a very good homogenous imaging region which is large enough for the mice foot tumor studies.

^{31}P MR Studies.--Fig. 5 shows the ^{31}P spectrum of a mouse foot tumor before the PDT. Figs. 6 and 7 show ^{31}P spectra recorded 20 min and 17 h after PDT. While small NTP peaks persist at the end of 20 min, the NTP peaks have disappeared at the end of 17 h indicating that all of the cancer cells have been killed. These results are similar to those of Ceckler et al. (1986). After three days, the RIF tumors on the mice feet stopped growing and began to shrink. After six days the tumors had disappeared totally.

Detection of ^{19}F Labeled Drugs in the Tumor.--Our preliminary studies on the ^{19}F labeled photosensitizer indicate that they can be non-invasively monitored by NMR spectroscopy. In Fig. 8 we show the first labeled photosensitizer peak five hours after direct injection of the labeled compound. This method of detecting and monitoring the photosensitizer is the first of its kind and this technique holds much promise for PDT research.

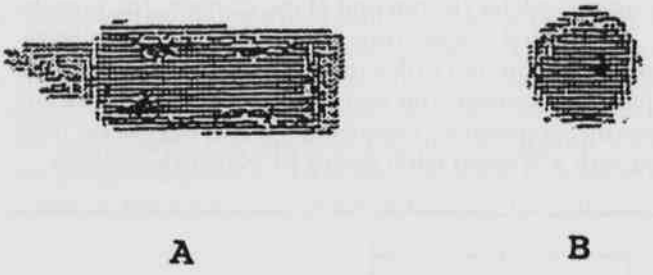

Fig. 3. Solenoid coil proton imaging
A). Axial
B). Sagittal

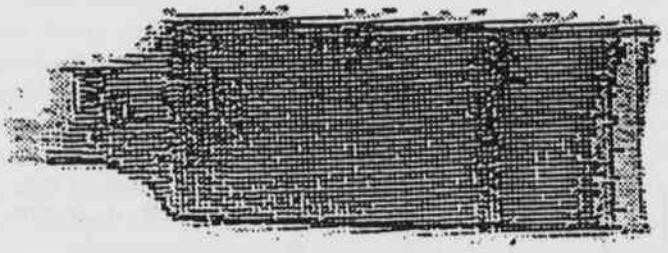

Fig. 4. The homogeneity of the Helmholtz coil proton imaging. Total length is about 1.5 cm, which is long enough for our project.

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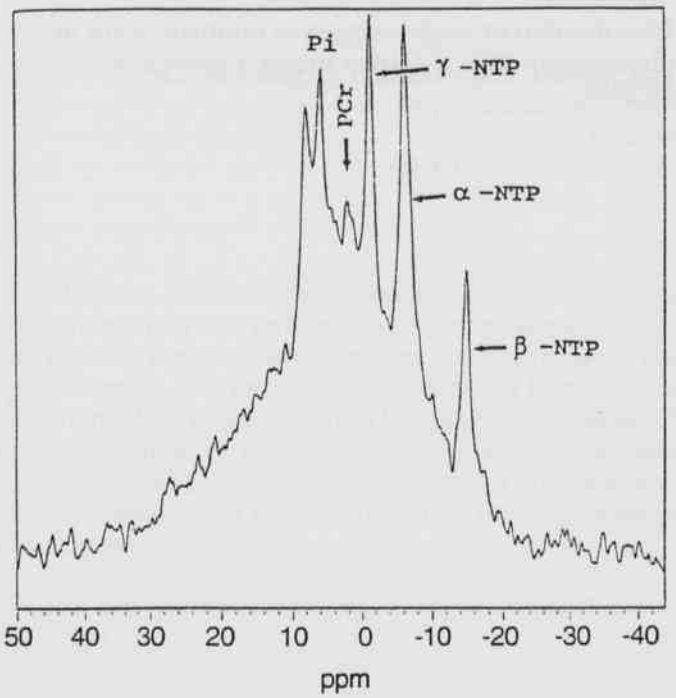

Fig. 5. ^{31}P-NMR spectrum of RIF tumor 24 hrs after the injection of 25 mg/kg of a photosensitizer, prior to PDT. Spectrum was obtained using 900 scans, a 90° pulse of 13 us, repetition time of 1 sec, and a total accumulation time 15 min.

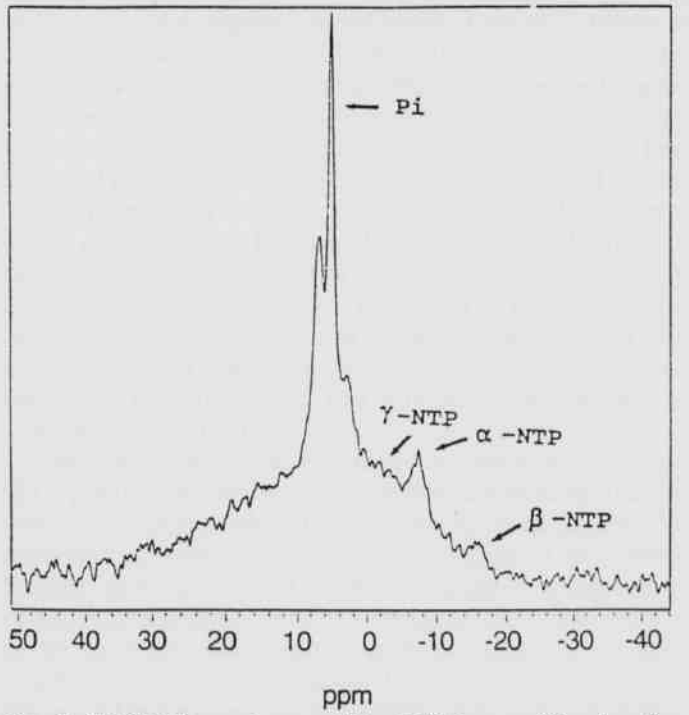

Fig. 6. ^{31}P-NMR spectrum of the RIF tumor 20 min after PDT, under same experimental conditions as prior to PDT.

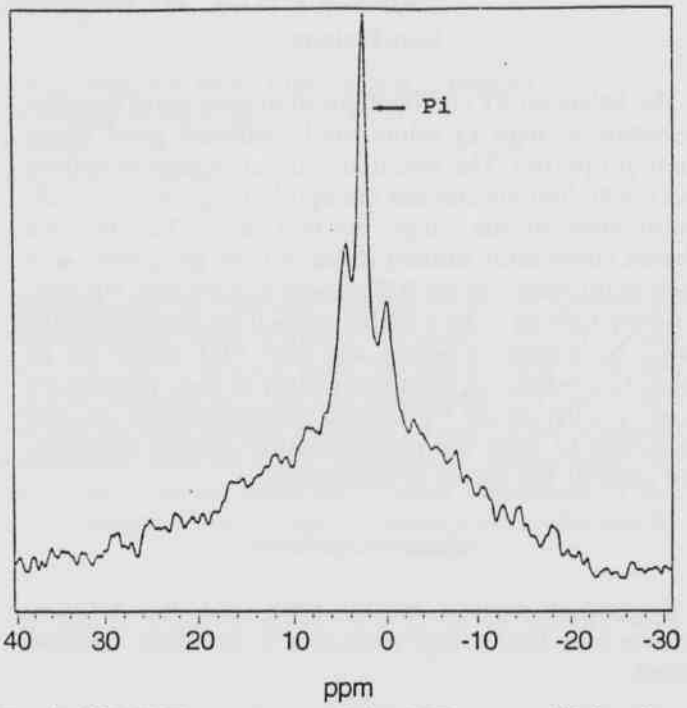

Fig. 7. ^{31}P-NMR spectrum of the RIF tumor 17 hr after PDT under same experimental conditions as prior to PDT.

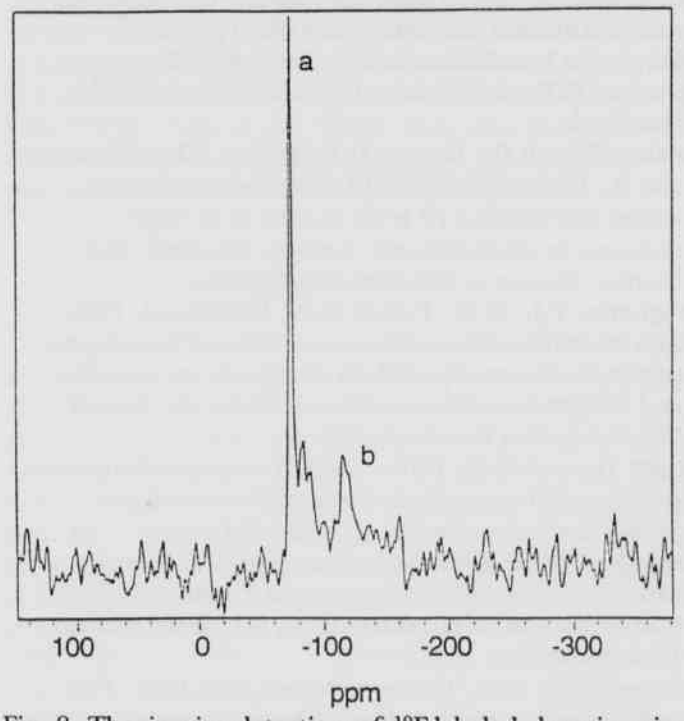

Fig. 8. The *in vivo* detection of ^{19}F-labeled drug in mice RIF tumor five hrs after injection. The parameters were: data size 8k, 7200 accumulations, repetition time 300 ms, a 10 μs 90° pulse, ^{19}F transmitter center at 188.26 MHz, total accumulation time of 36 min.

Conclusions

The balanced RF coil configuration gave good frequency response, high Q values, and exhibited good linear phase properties. The solenoid coil has enough sensitivity and the Helmholtz coil has enough homogeneity to study tumor sizes in the range 300-600 mm.[3] The fluorine labeled compound studied displayed the properties of a good photosensitizer on RIF tumors in mice feet. ^{31}p spectroscopy appears to be a useful method for monitoring the tumor bioenergetics before and after PDT which can be helpful in evaluating photosensitizers *in vivo*. Preliminary *in vivo* results on the ^{19}F labeled photosensitizers are very promising to study the photosensitizer uptake, retention, and possibly the extent of localization.

Acknowledgements

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