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DOE/ER/60892-4

**SYNTHESIS AND *IN-VIVO* DETECTION OF BORONATED
COMPOUNDS FOR USE IN BNCT**

Final Progress Report

August 1, 1989 - April 30, 1993

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August, 1993

PREPARED FOR THE U.S. DEPARTMENT OF ENERGY
UNDER GRANT NUMBER DE-FG05-893460892

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I. RESEARCH ACCOMPLISHMENTS

I am pleased to report that this D.O.E. Program at Tennessee has been a productive one. Twelve journal articles resulted from the work and sixteen scientific presentations were made; they are compiled in **Section II**.

A. **SYNTHESIS OF NEUTRON CAPTURE THERAPY AGENTS**

1. Carboranyl Precursors

Carboranes contain ten boron atoms in a three-dimensional space equivalent to a benzene ring; consequently, the carborane isomers can be utilized to prepare a variety of boron-rich agents for potential use in boron-neutron-capture therapy.

[Where $\bigcirc$ = BH, $\bullet$ = CH]

We developed synthetic methodology suitable for use with carboranes preparing amino acids and other physiologically active compounds of potential use in BNCT.

The methodology involves the conversion of simple carboranes into more complex, reactive organometallic reagents which can then be utilized to prepare agents which will target the nuclei of tumor cells. Specific examples include the projected syntheses of boron analogs of known intercalators such as Diazaquone (AZQ) which have been proven effectiveness in chemotherapy.

2. Gadolinium NCT Agents

We synthesized and carried out biodistribution studies of gadolinium labeled liposomes (GLL) which were developed in our laboratory. Gadolinium (like boron-10) has an excellent neutron cross section and is considered to be of potential use in neutron capture therapy. GLL are constructed by adding gadolinium based amphiphiles, such as the one illustrated on the next page, to liposome preparations.

Since liposomes can be selectively targeted to organs by coating them with various proteins or by sizing them, we prepared a series of liposomes and investigated their retention in the livers of Balb/c mice. The GLL tested were constructed by linking the Gd-DTPA head group to the stearyl chains via amide (Gd-DTPA-SA), ester (Gd-DTPA-SE), and thioester (Gd-DTPA-ST) bonds.

*[See Section IIa, articles 4, 5, 7, 8 and 9;
Section IIb, abstracts 2, 4, 7, 9]*

B. MULTINUCLEAR MAGNETIC RESONANCE IMAGING

Generation of MR images for fast relaxing nuclei such as boron is often hampered by the fact that the transverse relaxation times (T_2) are considerably shorter than the available time-to-echo (TE). The minimal TE achievable in MR imaging is limited by the need to apply phase-encoding and dephasing/rephasing read-out gradients which have finite rise times imposed by the hardware limitations. Currently, echo times of

2.5 ms are attainable using conventional spin-warp imaging techniques and it may be possible to shorten TE to approximately 1 ms by reducing the resolution requirements. However, boron-11 and boron-10 possess intrinsic transverse relaxation rates *in-vivo* which are so short that even after 1 ms most of the signal has already decayed, resulting in an unacceptably low signal.

Figure: *In Vivo* measurement of boron-11 transverse relaxation (T_2) in the rat.

To overcome problems due to the short T_2 of boron-11, we designed a back-projection technique from which we were able to generate the world's first boron-11 MRI image of a rat that had been infused with a BNCT agent. The spectra were acquired in the presence of variably orientated gradients and these spectra were used as input for reconstructing filtered back-projection images such as the one shown below.

Figure: First boron-11 image of a BNCT agent at a concentration used in preclinical BNCT studies which was obtained by reconstruction from 20 projections on a 64 × 64 matrix.

[See Section IIa, articles 1, 2, 3 and 4; Section IIb abstracts 1,2,3 and 4]

Though successful, back-projection boron-11 MRI is time consuming and provides only modest spatial resolution. We then developed an alternate imaging method for nuclei with very short T_2 relaxation times. As in backprojection, the technique utilizes the FID, rather than the echo signal, but involves only phase-encoding of the signal without frequency encoding. The method was tested by obtaining boron-11 images of aqueous boron phantoms, and was used to map the boron-11 distribution in an intact Fischer 344 rat infused with a therapeutic dose of dimeric sulfhydryl dodecaborane agent by our colleagues (Slatkin and Micca) at Brookhaven National Laboratory.

In the new method both spatial dimensions are phase-encoded by the usual amplitude cycling of gradients along the corresponding directions. The time during which these gradients are phase-encoding the signal is extremely short, 200 μ s. The time-domain signal is then Fourier transformed, in the usual manner, to yield an x-y image. An extension to three spatial dimensions can be achieved by adding a third phase-encoding gradient along the z-axis.

The minimal performance time of the proposed sequence is longer than that of a conventional spin-warp sequence since at least one scan for each resolution element is required. But this is not an obstacle for the imaging of boron-11 *in-vivo* since the necessary number of scans

needed for adequate signal averaging will usually exceed the minimal number of experiments required by the imaging process.

Figure: Pulse sequence for two-dimensional imaging of a species with a very short T_2 . The phase-encoding gradients are turned on prior to the rf pulse and turned off after collection of the first data point (A).

Imaging experiments were performed on a Gyrex 2T, 90cm bore, whole-body MR imaging unit operating at 26 MHz for boron-11 using a 6-turn solenoidal coil (10 cm diameter) adjusted to the size of the rat. All imaging experiments were conducted at ambient temperatures. The animals used were Fischer 344 male rats infused with sodium- μ -disulfide-bis(undecahydro-*clos*o-dodecaborate), $\text{Na}_4\text{B}_{10}\text{H}_{12}\text{S}_2$, at

Brookhaven National Laboratory; the rats were and shipped by air freight to Knoxville where the imaging experiments were performed.

FIGURE: Hydrogen (left) and boron-11 (right) MRI of a rat infused with a BNCT agent.

The new method possesses a number of advantages when compared to backprojection techniques. First, the natural linewidth of the signal (which is expected to be very broad due to the short T_2) does not degrade the spatial resolution since no frequency encoding is employed. Second, for the same reason, the images acquired by the method are free of artifacts and degradation resulting from magnetic field inhomogeneities, susceptibility variations and chemical shifts. (Both advantages are achieved without the need to employ gradients which are large compared to the prevalent interaction strengths in the spin system.)

Third, the analog filter can be set to a much smaller frequency cutoff, compared to backprojection, which results in a substantial improvement in signal-to-noise.

[See Section IIa, articles 8, 9, 10, and 11; Section IIb, abstracts 10, 11, 13, 14, 15 and 16]

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4. "Gadolinium Labeled Liposomes", G. W. Kabalka, M. Davis, and L. Huang, Society of Magnetic Resonance in Medicine Meeting, New York, NY (August, 1990).
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8. "Gadolinium-Labeled Liposomes Containing Various Amphiphilic Gadolinium-Diethylenetriamine-pentaacetic Acid Derivatives: Targeted MRI Contrast Agents", G. W. Kabalka, E. Buonocore, M. Davis, K. Muruyama, E. Holmberg, and L. Huang, Society of Magnetic Resonance in Medicine Meeting, San Francisco, CA (August, 1991).
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Columbus, OH (Aug, 1992).

IV. GRADUATE AND POSTDOCTORAL STUDENTS

Three postdoctoral students and three graduate students were
supported by this Department of Energy NCT Program.

A. Postdoctoral Student

Dr. Quang-Qiang Cheng
Dr. Charles A. Anderson
Dr. N. Kesavulu Reddy

B. Graduate Students

Mark A. Davis (Ph.D.)
George Hondrogiannis (Ph.D. candidate)
Zhe Wang (Ph.D.)

IV. FUTURE PLANS

The D.O.E. NCT program at The University of Tennessee has been fruitful. As outlined in **Section I**, we exceeded the objectives outlined in the original proposal and, in doing so, have developed new borane-based chemistry which we propose to use in the preparation of tumor-specific boronated NCT (BNCT) agents that will not affect neighboring healthy tissue. Even more significant is our development of the world's first method for detecting boron *in-vivo* (non-invasely) using whole-body magnetic resonance imaging. The project is continuing. New boron neutron capture agents are now being developed under DOE Grant #DE-FG05-93ER61610 during the period May 1, 1993–April 30, 1994.

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