DOE FINAL REPORT

Title: Carboranyl Oligonucleotides for Neutron Capture Therapy
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This proposal enabled us to synthesize and develop boron-rich nucleosides and oligonucleotide analogues for boron neutron capture therapy (BNCT) and the treatment of various malignancies. First, we determined the relationship between structure, cellular accumulation and tissue distribution of 5-*o*-carboranyl-2'-deoxyuridine (D-CDU) and its derivatives D-ribo-CU and 5-*o*-carboranyluracil (CU), to potentially target brain and other solid tumors for neutron capture therapy. Synthesized carborane containing nucleoside derivatives of CDU, D- and L-enantiomers of CDU, D-ribo-CU and CU were used. We measured tissue disposition in xenografted mice bearing 9479 human prostate tumors xenografts and in rats bearing 9L gliosarcoma isografts in their flanks and intracranially.

The accumulation of D-CDU, 1-(β-L-arabinosyl)-5-*o*-carboranyluracil, D-ribo-CU, and CU were also studied in LnCap human prostate tumor cells and their retention was measured in male nude mice bearing LnCap and 9479 human prostate tumor xenografts. D-CDU, D-ribo-CU and CU levels were measured after administration in mice bearing 9479 human prostate tumors in their flanks. D-CDU achieved high cellular concentrations in LnCap cells and up to 2.5 % of the total cellular compound was recovered in the 5'-monophosphorylated form. D-CDU cellular concentrations were similar in LnCap and 9479 tumor xenografts.

Studies in tumor bearing animals indicated that increasing the number of hydroxyl moieties in the sugar constituent of the carboranyl nucleosides lead to increased rate and extent of renal elimination, a decrease in serum half-lives and an increased tissue specificity. Tumor/brain ratios were greatest for CDU and D-ribo-CU, while tumor/prostate ratios were greatest with CU. CDU and D-ribo-CU have potential for BNCT of brain malignancies, while CU may be further developed for prostate cancer.

A method was developed for the solid phase synthesis of oligonucleotides containing (*o*-carboran-1-yl-methyl)phosphonate (CBMP) internucleotide group. Unmodified phosphodiester linkages were formed using a standard *β*-cyanoethyl cycle and automated DNA synthesizer. Modified CBMP internucleotide linkage was produced using the phosphotriester method and 5'-*O*-monomethoxytritylthymidine 3'-*O*-[(*o*-carboran-1-yl-methyl)phosphonate] monomer. Several dodecathymidylic acids bearing modification at 3'- or 5'-end, or in the middle of oligonucleotide chain were synthesized. The resulting oligomers are being characterized by reverse phase high-pressure liquid chromatography (RP-HPLC), electrospray ionization mass spectrometry (ESI-MS), ultraviolet spectroscopy (UV), and circular dichroism (CD).

In collaboration with Cornell University, we employed a secondary ion mass spectrometry (SIMS) based subcellular isotopic imaging technique of ion microscopy for evaluating 4 carboranyl nucleosides. Nucleosides synthesized by our group, including CDU, HMCDU, CTU, and CFAU were tested for their boron delivery to the nuclear and cytoplasmic compartments of U251 human and F98 rat glioma cells. Quantitative SIMS analysis of boron was performed in cryogenically prepared cells. For all drugs, the cell cytoplasm revealed significantly higher boron than the nucleus. However, the boron partitioning between the cell nucleus and the nutrient medium indicated 6.4-10.6 times higher boron in the nucleus. The results suggested that these novel carboranyl nucleosides should provide efficient BNCT agents that accumulate in malignant cells and the need for further evaluations *in vitro* and in animal models.

Efficacy studies for certain tumor selective boron compounds suggested that accumulation is cell cycle dependent, leading to inherent drug distribution heterogeneity within the targeted cell population. Therefore, investigating the effect of the cell cycle on the accumulation and egress heterogeneity in human-derived glioma cell line (U-251) is an important factor in characterizing the pharmacokinetics of a potential BNCT agent. FACS analysis showed an ~80% synchronization for each target phase with thymidine (0.25 mM). Kinetic studies indicated that synchronization remains up to 4 hr in the S phase and indefinitely in the G_1 phase. These observations provided the foundation for experimental data on the accumulation and egress of radiolabeled D-CDU in synchronized U-251 cells. Cells

synchronized in the S-phase accumulated significantly higher amounts of *nido* and *closo*-CDU and their respective 5'-monophosphates.

Unilamellar liposomes of specific size and composition can be used as carriers of boron therapeutical concentrations to neoplastic cells The effect of D-CDU on phospholipid phase behaviour and on phospholipid dynamics were investigated using Electron Spin Resonance (ESR) spectroscopy. Fatty acid spin labels (*n*-doxylstearic acid, *n*-DSA) and the nitroxide-labeled cholesterol analogue cholestane (CSL) were incorporated into liposomes built up with saturated distearoyl phosphatidylcholine (DSPC), unsaturated egg yolk phosphatidylcholine (EPC) and mixtures of DSPC/cholesterol (55-45%mol), EPC/cholesterol (55-45%mol). The CDU content in the liposome dispersion was determined by measuring UV absorption at 274 nm. The data clearly showed that CDU insertion has a significant influence on the lipid bilayer structure and temperature dependent phase behaviour. The analysis of the ESR lines by spectral simulation supports the increase of membrane fluidity and the ordering effect of the carboranyl-nucleoside into the lipid packing over the whole temperature range. The differences in the interaction and organization between the boronated nucleoside and both the cholesterol free-, and the cholesterol-containing liposomes were also studied.

Of significance was the finding that D-CDU was not toxic to rats injected intraperitoneally with up to 150 mg/kg. Based on these studies, D-CDU was evaluated as a neutron capture therapy agent using rats bearing stereotactically implanted intracranial 9L tumors at single intraperitoneal doses of 30 mg/kg and 150 mg/kg of D-CDU (20% ^{10}B enriched), given 2 hr before irradiation with thermal neutrons. Boron concentrations in tumors 2 hr after dosing were 2.3 ± 1.6 and 7.4 ± 1.3 μg B/g tissue (mean ± SD), corresponding to tumor/brain ratios of 11.5 ± 3.6, and 6.8 ± 2.0 for the low and high doses, respectively. All untreated animals died within 28 days, while half survived at days 32, 55 and 38, for groups receiving neutrons only, 30 mg/kg, and 150 mg/kg D-CDU, respectively. Odds ratios of all treatment groups differed significantly from the untreated group ($p < 0.002$; logrank test). The median survival time for the 30 mg/kg treated group, but not the 150 mg/kg group, was significantly longer than for rats treated with neutrons only ($p = 0.036$), which may correlate with the decreased tumor selectivity for D-CDU observed at the higher dose. This work provided proof-of-principal data in a relevant animal model that the BNCT concept should also work in humans. Clearly, to take these concepts to the next steps, additional pharmacodynamic studies to determine optimal dosing strategies for D-CDU should be performed as well as the development of improved neutron beams for use in human clinical studies.

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