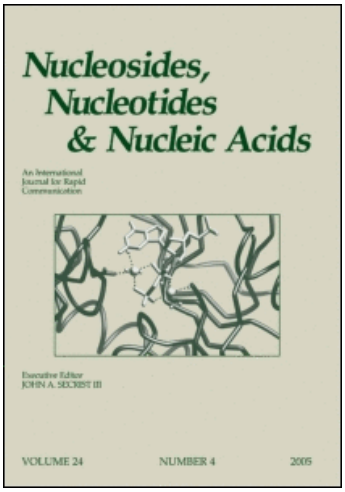

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Tissue Disposition of 5-*o*-Carboranyluracil—A Novel Agent for the Boron Neutron Capture Therapy of Prostate Cancer†

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ABSTRACT

The carboranyl nucleotides β-D-5-*o*-carboranyl-2′-deoxyuridine (D-CDU), 1-(β-L-arabinosyl)-5-*o*-carboranyluracil (D-ribo-CU) and the nucleotide base 5-*o*-carboranyluracil (CU), were developed as sensitizers for boron neutron capture therapy (BNCT). A structure activity study was initiated to determine the agent most suitable for targeting prostate tumors. Cellular accumulation studies were performed using LNCaP human prostate tumor cells, and the respective tumor disposition profiles were investigated in male nude mice bearing LNCaP and 9479 human prostate tumor xenografts 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. In vivo concentrations of D-CDU were similar in LNCaP

†In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.

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and 9479 tumor xenografts. Studies in 9479 xenografted bearing mice indicated that increasing the number of hydroxyl groups in the sugar moeity of the carboranyl nucleosides corresponded with an increased rate and extent of renal elimination, shorter serum half-lives and an increased tissue specificity. Tumor/normal prostate ratios were greatest with the nucleoside base CU. These studies indicate that similar nucleoside analogues and bases may have different tissue affinities and retention properties, which should be considered when selecting agents for sensitizing specific tumors for eventual BNCT treatment. CU was found to be the most suitable compound for further development to treat prostate cancer.

Key Words: Boron neutron capture therapy; Nucleosides; Prostate tumors.

INTRODUCTION

After skin cancer, prostate cancer is the second most frequently diagnosed cancer in the US male population. The American Cancer Society estimated that there will be over 198,000 new cases of prostate cancer diagnosed and more than 31,500 deaths in the US during 2001.[1] Boron neutron capture therapy (BNCT) is a binary treatment modality based on the selective uptake of sufficient boron isotope ^{10}B into tumor cells ($\sim 10^9$ atoms per cell or 5–30 ppm), followed by irradiation with epithermal neutrons. The resulting nuclear capture and fission reactions produce α particles and ^7Li nuclei which have high linear energy transfer and path lengths of 9 and 5 μm, respectively, that are lethal to approximately one cell diameter.[2–6] Although surgery and radiotherapy have been successful,[7] BNCT may offer theoretical advantages, especially if ^{10}B delivery agents are developed that demonstrate a high specificity for tumor tissue.

Despite considerable drug development efforts only three ^{10}B delivery agents have received approval from the United States Food and Drug Administration for testing in humans. These include sodium borocaptate (BSH), a carborane derivative containing ten ^{10}B atoms per molecule, boranophenylalanine, an amino-acid containing one ^{10}B atom per molecule and the polyhedral borane dianion $[closo\ B_{10}H_{10}]^{2-}$ (GB-10), all of which have limitations with regard to tumor specificity.[8] Provided differences exist in the accumulation and/or depletion rates ^{10}B delivery agents in tumors and surrounding tissue, the binary nature of BNCT could be exploited by administering the neutrons when the tumor to normal tissue or tumor the blood ratio is maximal.[9] Prostate tumors are potential candidates for BNCT since they are readily accessible to a neutron beam (1.0–1.5 cm from the surface).[7,10] Therefore, provided boron delivery agents are developed that preferentially accumulate in the tumor relative to surrounding tissues such as the bladder and rectum, damage to surrounding tissue could be minimized.

Like the endothelial layer that surrounds the brain, the layer of sertoli cells that surround the prostate gland serves as a protective barrier that is impermeable to many compounds.[7,11,12] During tumor development, these barriers to drug penetration in the region of the tumor are compromised relative to surrounding non-tumor tissue. Therefore, compounds similar to those being developed for brain tumors may have potential to treat prostate tumors. β-D-5-*o*-carboranyl-2′-deoxyuridine (D-CDU) is a lipophilic nucleoside derivative (octanol/water partition coefficient = 3.05×10^3) that

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shows potential for the treatment of brain tumors with BNCT.[13–20] The cellular accumulation of D-CDU is rapid, non-concentrative and not sensitive to the potent nucleoside uptake inhibitors nitrobenzylthioinosine and dipyridamole or by D-thymidine. However, the rapid accumulation of D-CDU was strongly inhibited by the nucleoside base uptake inhibitor papaverine.[20,21] Therefore, the mechanism of cellular uptake may resemble other modified nucleoside derivatives such as acyclovir and 5-fluorouracil where accumulation is at least partially mediated by a nucleoside base uptake route.[22–24] D-CDU-5′-monophosphate (D-CDUMP) has been isolated from cells treated with D-CDU and is formed following incubation in the presence of recombinant thymidine kinases.[14,15,19,20] That phosphorylation occurs intracellularly and accumulation is inhibited by papaverine, suggests that D-CDU can enter cells. D-CDU-MP is negatively charged and does not egress cells easily. Phosphorylation of nucleosides may be enhanced in rapidly dividing cells in tumors. Therefore, phoshorylation may be a mechanism for increasing the tumor selectivity of compounds related to D-CDU. D-CDU is non-toxic against exponentially growing human lymphoblastoid CEM cells, human U-251 glioma cells, and rat 9L glioma cells in the absence of neutron irradiation, which should limit toxicity to tissues outside the field of neutron therapy. The therapeutic ratio of BNCT is proportional to the concentration ratio of ^{10}B in the tumor relative to surrounding tissue at the time of neutron irradiation.

A BNCT study using D-CDU demonstrated a significant increase in the median survival times of rats bearing 9L intracranial brain tumor isografts.[15] Furthermore, the related derivatives 1-(β-L-arabinofuranosyl)-5-*o*-carboranyluracil (ara-CU), 1-(β-L-arabinosyl)-5-*o*-carboranyluracil (D-ribo-CU) and the nucleoside base CU have altered protein binding and cellular accumulation properties.[18] Therefore, in vivo studies were performed to compare the relative tissue specificities of D-CDU, D-ribo-CU and CU, in order to select the agent most favorable for targeting prostate tumors.

EXPERIMENTAL

Chemical synthesis. The synthesis of D-CDU, CU and D-ribo-CU have been previously reported.[16,18] D-CDU, D-ribo-CU and CU were radiolabeled with tritium (^3H) at the 6-position and on the acidic proton of the carboranyl moiety by Moravek Biochemicals, Inc. (Brea, CA), by hydrolysis of the lithium derivative with carrier-free tritiated water. The specific activities of CU D-ribo-CU and D-CDU were 59.2, 1.2, and 1.8 Ci/mmol, respectively. ^{14}C labeled D-CDU (55 mCi/mmol) was synthesized using 5-iodo-2′-deoxyuridine-2-^{14}C. The chemical structures of D-CDU, D-ribo-CU and CU are shown in Fig. 1.

Cell culture. LNCaP human prostate cancer cells were obtained from the American Type Culture Collection (Monassis, VA) and grown as a monolayer in RPMI 1640 medium (Cellgro, Hernden, VA) at 37°C in 5% CO_2 in air, and in 75 cm^2 tissue culture flasks (Corning Scientific Products, NY). Ten percent heat inactivated fetal bovine serum (Grand Island Biological Company, Gaithesburg, MD), 2 mM L-glutamine (Grand Island Biological Company, Gaithesburg, MD) and antibiotics (penicillin 100 IU/ml and streptomycin 100 μg/ml, Cellgro/Mediatech, Hernden, VA) were added to the medium (maintenance medium). The doubling time of LNCaP cells

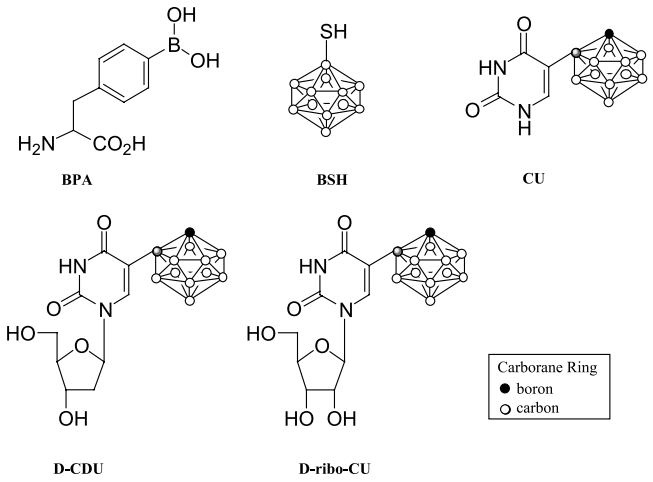

Figure 1. Chemical structures of the carboranyl compounds: BPA, *p*-boranophenylalanine; BSH, borocaptate sodium; CU, 5-*o*-carboranyluracil; D-CDU, β-D-5-*o*-carboranyl-2′-deoxyuridine; D-ribo-CU, 1-(β-L-arabinosyl)-5-*o*-carboranyluracil.

was about 18 h. 9437 cells were generously supplied by Dr. Thomas Keane, of the Department of Urology, Emory University School of Medicine. This hormone-independent prostate cancer cell line was passaged only as an in vivo xenograft.

Accumulation of D-CDU in LNCaP human prostate cells. Cell accumulation and egress studies were performed in LNCaP human prostate cancer cells grown in monolayer in 6-well plates (Nunc, Intermed, Naperville, IL) to 90% confluence ($\sim 0.33 \times 10^6$ cells per ml) in 2 ml of maintenance medium. The medium was replaced with 2 ml of fresh pre-warmed minimal essential medium (MEM) containing 10 μM ^{3}H-D-CDU (specific activity 2.5 Ci/mmol, 1,000 dpm/pmol) and 2% heat inactivated fetal bovine serum (FBS) and incubated at the above conditions (time = 0 for accumulation experiments). For egress studies, cells were treated with D-CDU for 2 h, after which the media was removed and the cells resuspended in fresh medium containing 2% FBS (time = 0). At designated times, cells were placed on ice, washed twice with ice-cold PBS followed by lysing using 400 μl of 60% methanol in water. The wells were washed again with a further 400 μl of 60% methanol in water, and the combined lysates stored at -70°C until they were assayed using high performance liquid chromatography (HPLC).

HPLC was used to measure the total amount and the fraction of D-CDU that was phosphorylated in LNCaP human prostate tumor cells.[20] LNCaP samples were thawed at 4°C and centrifuged for 10 min at $16{,}000 \times g$. The samples were dried at room temperature using a Speed Vac centrifuge dryer (Model SC110, Savant Instruments Inc., Farmingdale, NY). The dry extract was dissolved in 40 μl of 100 mM tris buffer containing 20 mM magnesium chloride and divided into two aliquots. To dephosphorylate possible nucleotides of D-CDU, 22 units of alkaline phosphatase from calf intestinal mucosa (EC 3.1.3.1, type XXX-A, Sigma, Saint Louis, MO) was added to each aliquot, prior to incubation at 37°C for 2 h. The solution was then diluted to

100 μl with buffer and injected into the HPLC. A gradient consisting of mobile phase A (0.05 M triethylamine adjusted to pH 7 with acetic acid in water) and mobile phase B (50% acetonitrile diluted with 2-fold buffer A) was used. The starting solvent ratio was 5% solvent B which increased to 30% solvent B over 40 min. A Whatman Partisphere C_{18}, 5 μm column (Whatman, Clifton, NJ) was used. The CDU metabolites were detected by UV at 270 nm and ^{3}H was monitored using a radiomatic Flo-one *Beta* detector (Packard, Downers Grove, IL). The retention times for D-CDU-5′-diphosphate, the *nido*D-CDU derivative, D-CDU-5′-monophosphate and D-CDU (*closo* form) were validated at 12, 14, 24 and 37 min respectively, using samples of these compounds which were synthesized in our laboratory.[18]

Single dose toxicity of D-CDU in male nude mice. Non-radioactive D-CDU was injected intraperitonealy (i.p.) into male nude mice (without tumors). Doses injected were 0, 30, 60 120, 150 mg/kg (n = 3 per group) and 180 mg/kg (n = 6). Mice were weighed and observed for toxicity over the next 30 days. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the Veterans Medical Center, Atlanta GA.

Disposition studies in male nude mice bearing LNCaP and 9479 xenografts. Male nude mice were implanted with human LNCaP and 9479 prostate tumor xenografts in their flanks by sub-cutaneous injection of $\sim 10^{7}$ cells suspended in 0.2 ml PBS. Xenografts were grown until they reached the required diameter (see below).

Accumulation in vivo. A comparative in vivo accumulation study of D-CDU was performed in mice bearing human 9437 xenografts (n = 3 per time point) and in mice bearing xenografts of the hormone-dependent LNCaP cell line that had been passaged in vitro (n = 2 per time point). When tumors reached ~1.5 cm in diameter, mice were given i.p. injections of 5 mg/kg radiolabeled D-CDU dissolved in 100 μl dimethylsulfoxide. The specific activities of the D-CDU injections were 8 μCi of ^{14}C, and 15 μCi of ^{3}H for the 9437 and LNCaP bearing mice, respectively. Mice bearing 9437 xenografts were euthanized at 0.5, 1, 3 and 6 h, using an i.p. injection of ketamine and xylazine (at 0.39 and 0.039 g/kg, respectively). The total radiolabeled compound was extracted and measured from the tumor, prostate, bladder, rectum, brain and abdominal fat. A less detailed study was performed using mice bearing LNCaP xenografts, which were sacrificed at 1, 2 and 3 h after D-CDU injection. The total radiolabeled compound was measured in the tumor, serum and brain for comparison with the 9437 xenografts.

To determine the optimal tumor size for comparing compounds related to D-CDU in 9479 xenografts, tumors were grown until their respective diameters reached 0.5, 1.5 and 2 cm in diameter (3 per group). Animals were euthanized 1.5 h after an i.p. injection of ^{14}C-labeled D-CDU (5 mg/kg, dissolved in 100 μl DMSO, 8 μCi ^{14}C). The concentrations of total ^{14}C-labeled D-CDU in the various tumor groups was then measured (see below). Further experiments were performed in animals bearing 9437 tumor xenografts.

Tumor and tissue concentrations of D-ribo-CU and CU were compared to those observed with D-CDU in 9437 tumor (~1.5 cm diameter) bearing mice following i.p. injections at a constant 5 mg/kg dose. The sampling times and experimental design for

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the D-ribo-CU and CU experiments were the same as the D-CDU experiment (see above). The amount of radioactive compound injected per animal was 15 μCi of ^{3}H for the D-ribo-CU, and CU dissolved in 100 μl DMSO.

Tissue samples were weighed, rinsed with phosphate buffered saline and suspended in 1 M NaOH for 18–60 h with constant shaking. The suspensions were then neutralized with an equal volume of 1 M HCl, and extracted overnight in 12% methanol/water in a final volume of 1.6 ml in a shaker, and centrifuged to clarify supernatant. One ml of supernatant was combined with 15 ml Ecolite and measured for ^{3}H and ^{14}C using a scintillation counter. Results were expressed as pmol of compound per mg of tissue wet weight using the specific activities of the respective compound. Since the carboranyl moiety and the ^{3}H and ^{14}C radiolabels were located at stable positions of the molecules, the radioactivity was considered a direct indicator of boron accumulated. Previous studies have indicated similar boron concentrations estimated using radiolabeled D-CDU and direct measurements using boron atomic emission spectroscopy (DCP-AES).[15]

Pharmacokinetics in male nude mice 9479 human prostate xenografts. The plasma concentrations of D-CDU, D-ribo-CU and CU measured at 0.5, 1, 3 and 6 h, were fitted to the pooled serum data from all mice, using the non-compartmental pharmacokinetics routine of WinNonlin (Ver. 1.5, 1997, SCI INC, NC), under the assumption of a non-i.v. route of administration. The terminal half-life ($t_{1/2,\beta}$, h) was estimated using data from 1 to 6 h. The mean residence times (h) were calculated as the ratio between the cumulative areas under the serum concentration versus time curve (AUC, μM × h) and the area under the first moment curve (AUMC, μM × h^2).[25] Other parameters estimated include the respective volumes of distribution during the terminal elimination phase (V_β) divided by the unknown fraction (F) of total dose absorbed into the circulation (V_β/F, l/kg) and the total plasma clearance (Cl_T) divided by F (Cl_T/F, l/h/kg).

RESULTS AND DISCUSSION

The accumulation of carboranyl nucleosides D-CDU, D-ribo-CU and the nucleoside base CU were studied in vitro and in vivo using radiolabeled compound. Previous studies with radiolabeled D-CDU have shown that when the radiolabels were inserted in stable positions within the molecule, radiological measurements are similar to direct measurements of boron in tissues using direct current plasma-atomic emission spectroscopy (DCP-AES).[15] Furthermore, unlike DCP-AES that cannot distinguish between the boron delivery agent and its metabolites, the use of compound radiolabeled in a chemically stable position permitted the parent *closo*-containing compounds to be distinguished from the less lipophilic *nido*-compound, and the nucleosides to be differentiated from their phosphorylated metabolites using reverse phase HPLC with radiomatic detection.[15,20]

The accumulation of D-CDU in LNCaP cells in vitro was rapid and achieved pseudo-equilibrium conditions in less than 10 min. After 2 h incubation, the cellular concentrations (mean ± SD) of D-CDU were $4.08 \pm 0.38 \times 10^2$ pmol/10^6 cells, of which 2.09 ± 1.65 percent was in the 5′-monophosphate form. The half-life of egress for

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the D-CDUMP from LNCaP cells was 0.9 h as measured over a 48 h period (r^2 of the natural log of D-CDUMP versus time = 0.87). Therefore, the cellular pharmacology of D-CDU in human prostate cancer cells was similar to that previously measured in human lymphoblast CEM cells, U-251 human brain tumor cells and rat 9L glioma cells.[15,20]

A previous in vitro study demonstrated that altering the number and orientation of hydroxyl moieties in D-CDU derivatives altered the cellular accumulation and protein binding.[18] Therefore, in vivo studies were performed to determine whether, D-ribo-CU (the most hydrophylic agent), D-CDU and the nucleoside base CU (the most lipophilic agent), had different tissue affinities which could be beneficial for the more selective delivery of ^{10}B to tumors at an equal dose of 5 mg/kg.

The concentrations of D-CDU were measured 1.5 h after an i.p. dose of 5 mg/kg, in two tumor xenografts derived from the hormone dependent human LNCaP prostate cell line (1.5 cm diameter) were 90 and 47.3 ng/g (wet weight), while those measured in 9437 xenografts of the same diameter were 67 ± 13 ng/g (mean ± SD, n = 3), suggesting that the average tumor dispositions observed were representative of more than one prostate cell line.

The effect of tumor size on the disposition of D-CDU was studied in mice bearing 9437 tumor xenografts (1.5 to 2.0 cm). Concentrations of ^{10}B resulting from D-CDU at 1.5 h following i.p. injection were similar for tumors of diameter 0.5 cm (472 ± 80 ng/g wet weight, mean ± SD) and 1.5 cm (432 ± 32 ng/g wet weight). However, lower concentrations were observed in tumors with diameters > 2.0 cm (280 ± 57 ng/g). This probably resulted from the greater degree of necrosis and apparent decreased cellularity observed in the larger tumors. Therefore, comparative studies of D-ribo-CU, D-CDU and CU were performed in animals with tumors with diameters of ~1.5 cm.

Figs. 2–4 depict the concentrations of ^{14}C labeled D-CDU, ^{3}H-D-ribo-CU and ^{3}H-CU, respectively in the organs of nude mice bearing 9437 tumors in their left flank following a 5 mg/kg ip. Early serum concentrations (t < 0.5 h) of D-CDU and D-ribo-CU were higher than those of CU, suggestive of a smaller volume of distribution for the more hydrophilic agents. CU, the most lipophilic of the series had the greatest affinity for fat and nerves and the least affinity for bladder tissue. The elimination profiles of D-CDU, D-ribo-CU and CU from the serum are shown in Fig. 3. Tumor/serum, tumor/prostate and tumor/bladder ratios versus time indicate a time lag before equilibration between the prostate tumor and surrounding tissues (Table 1). Tumor/bladder ratios of D-ribo-CU and CU at 3 h, were 10 and 30 fold higher, respectively compared to D-CDU. The concentrations of D-CDU, D-ribo-CU and CU at 3 h in the serum, tumor prostate and brain are shown in Fig. 4.

Early serum concentrations of D-CDU and D-ribo-CU (t < 0.5 h) (Fig. 3) were higher than those of CU, in agreement with a smaller initial distribution volume for less lipophilic agents.

As shown in Figs. 2 and 3, D-CDU and D-ribo-CU were cleared from serum more rapidly ($t_{1/2,\beta}$ = 1.4 and 1.2 h and MRT = 1.6 and 1.2 h, respectively) with a larger percentage of accumulation into the urinary bladder compared to CU ($t_{1/2,\beta}$ = 2.2 and MRT = 3.1 h). The terminal $t_{1/2}$ of 1.4 h for D-CDU measured in mice was similar to previously published 1.3 h $t_{1/2}$ in rats.[26] The respective V_β/F and Cl_T/F values were 0.82, 4.76 and 3.17 l/kg, respectively and 0.49, 2.36 and 1.0 l/h/kg, respectively for D-ribo-CU, D-CDU and CU. This suggests that D-ribo-CU may distribute into a smaller tissue volume and be eliminated more rapidly by the kidney than D-CDU and CU.

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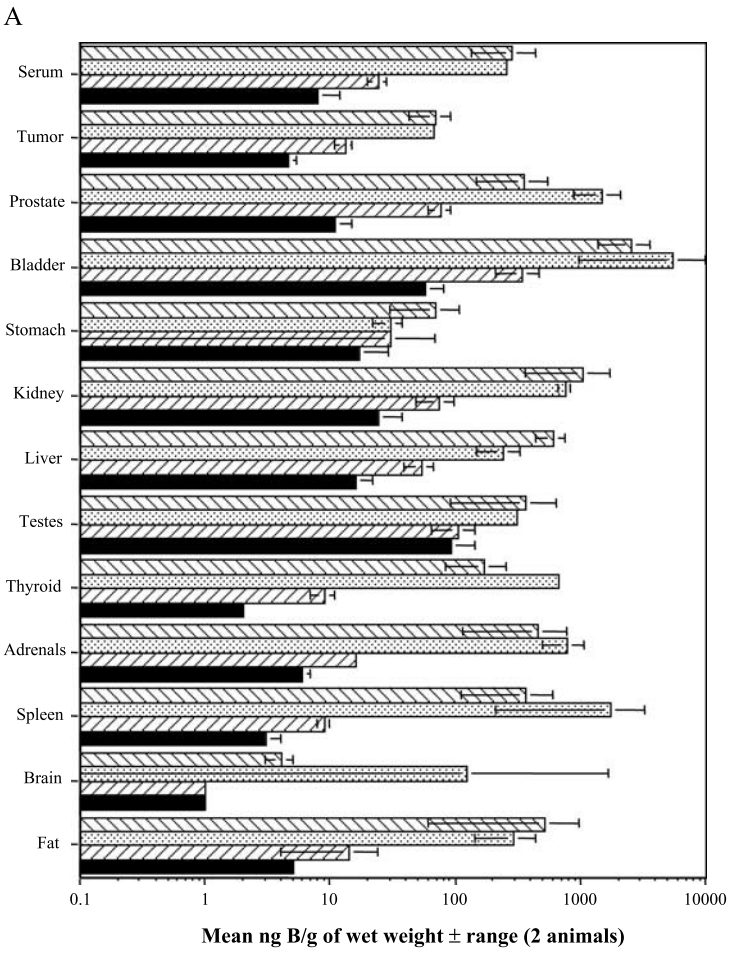

Figure 2. Organ concentrations of D-CDU (A, 2 mice per time point), D-ribo-CU (B, 3 mice per time point) and CU (C, 3 mice per time point) in nude mice bearing 9437 tumors in their left flank and given 5 mg/kg doses of radiolabeled compound. Bars represent tissue levels obtained at 0.5 (▨), 1 (⊡), 3 (◪) and 6 (■) h, respectively. An additional time point 0.25 h (□) was also obtained for CU (C). Since there are ten ^{10}B atoms per molecule of compound, each ng of ^{10}B/g of tissue corresponds to 0.01 nmol of compound/g of tissue.

Absolute values for V_β and Cl_T in the mice were not computed in this study, since i.v. data were not available. Therefore, a direct comparison could not be made regarding differences in the corresponding V_β and Cl_T in mice and rats from this study. The values for V_β and Cl_T in the rats following an i.v. injection (F = 1) were 0.70 l/kg and 0.69 l/h/kg.[26] A direct comparison of V_β and Cl_T values of D-CDU could not be made between the previously reported i.v. study in rats and this study i.p. study in mice, since D-CDU was injected i.p. in mice using a DMSO vehicle at a concentration that exceeded its aqueous solubility. Therefore, complete absorption from the peritoneal compartment following the i.p. injection could not be assumed (F < = 1).

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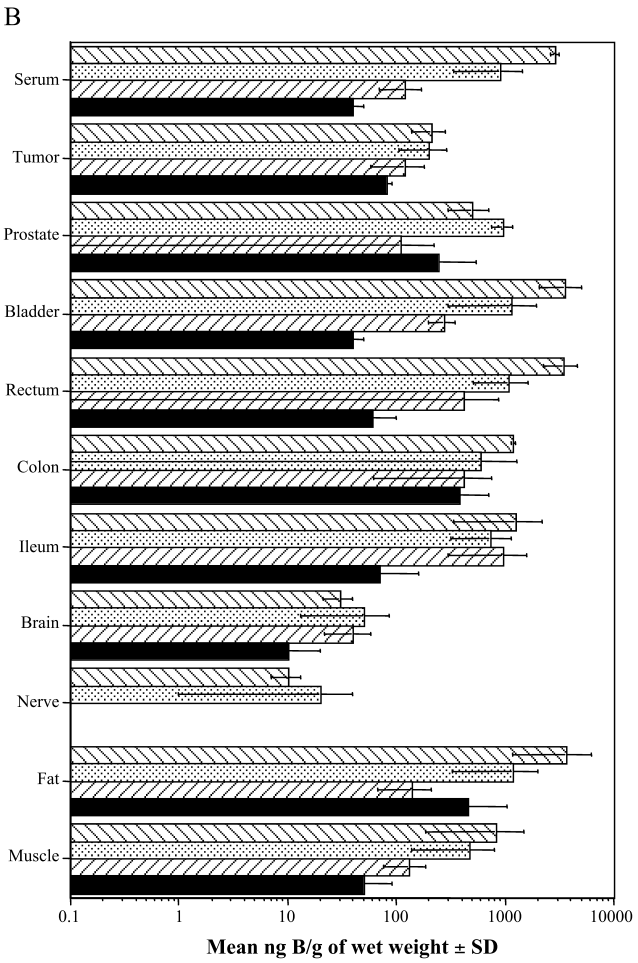

Figure 2. Continued.

(continued)

Neutron irradiation should be administered when the ^{10}B concentrations in the prostate tumor are sufficiently high to achieve fission in the tumor and lower in the non-targeted surrounding tissue such as the bladder to minimize side effects.[9] The ratio of tissue concentration versus time in Table 1 indicates a slower egress rate of the D-CDU analogues from tumors relative to prostate. The precise mechanism for the relatively slow clearance has not been elucidated. However, it may result from the physiological constrains of tumors which are limited by their blood supply and their lack of lymphatic drainage.[27] Based on data presented in Table 1, it may be appropriate to administer neutron irradiation 2–3 h after dosing, since tumors would retain significant drug levels at that time while the levels in surrounding tissues would have decreased.

Previous studies have demonstrated that D-CDU crosses the compromised blood–brain-barrier of isografted rat 9L brain tumors with a tumor/brain ratio of > 10 at 2 h,

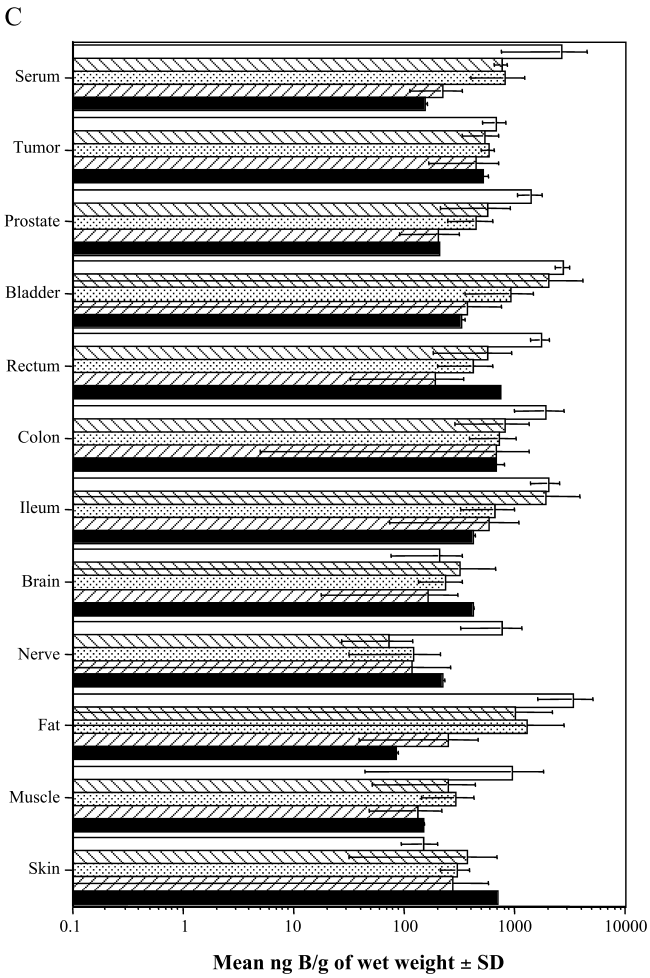

Figure 2. Continued.

suggesting that this agent has potential to treat brain cancer.[15] Concentrations achieved in the 9437 tumor xenografts were similar to those reported in 9L tumors in rats treated with D-CDU. The more hydrophilic agents D-CDU and D-ribo-CU cross the intact blood–brain-barrier less readily than CU. Although CU achieved higher tumor concentrations than D-CDU, it also produced a lower differential between brain and non-brain tissues (Fig. 2). Since brain tumors often result in a compromised blood–brain barrier, drug tumor levels may approach concentrations similar to non-brain tissues. Thus, a lower brain tumor/brain concentration ratio would be expected for CU compared to the D-CDU and D-ribo-CU,that penetrate the barrier less efficiently. A decreased relative survival and a concomitant decrease in tumor/normal brain ratio at higher doses were observed in rats bearing 9L isografts and treated with 20% ^{10}B enriched D-CDU and neutrons. This could result from damage to the normal brain when boron concentrations accumulate to levels sufficient to sustain a neutron reaction.[15]

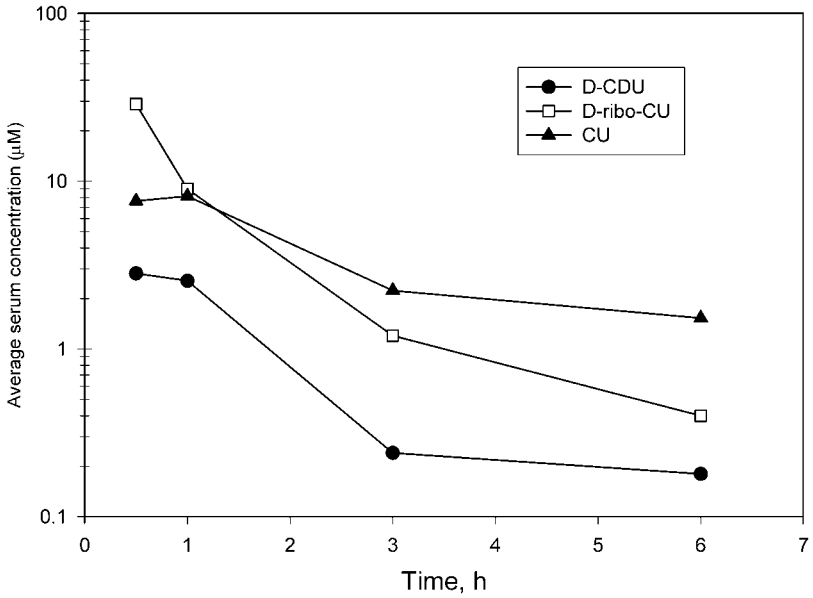

Figure 3. Serum concentrations of D-CDU, D-ribo-CU and CU, in nude mice bearing 9437 tumors in their left flanks and given 5 mg/kg i.p. doses of the respective compound. Since there are ten ^{10}B atoms per molecule of compound, 1 μM compound corresponds to 100 ng ^{10}B per ml.

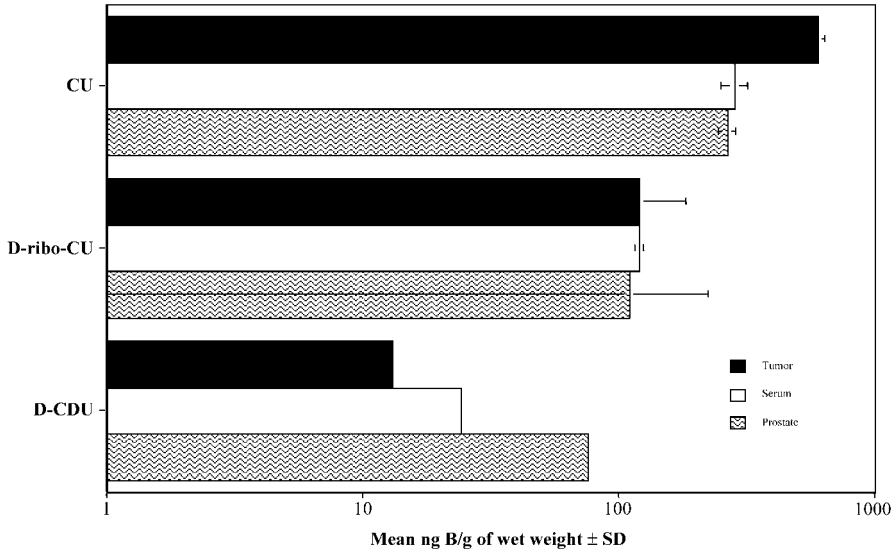

Figure 4. Retention of CDU, D-ribo-CU and CU at 3 h in the serum, tumor, and prostate tissues of nude mice bearing 9437 tumors in their left flanks and given a 5 mg/kg i.p. dose of the respective compounds.

Table 1. Mean organ concentration ratios for radiolabeled ^{14}C-D-CDU and ^{3}H-CU and ^{3}H-ribo-CU in mice bearing 9479 human prostate tumor xenografts.

Time after drug administration	^{3}H-CU (n = 3)				^{14}C-D-CDU (n = 2)				^{3}H-D-ribo-CU (n = 3)			
	0.5 h	1 h	3 h	6 h	0.5 h	1 h	3 h	6 h	0.5 h	1 h	3 h	6 h
Tumor/Serum	0.69	0.7	2.0	3.4	0.25	0.3	0.5	0.6	0.07	0.22	1.0	2.0
Tumor/Bladder	0.26	0.6	1.2	1.6	0.027	0.01	0.04	0.09	0.06	0.18	0.4	2.0
Tumor/Prostate	0.93	1.4	2.2	2.5	0.19	0.5	0.2	0.5	0.42	0.2	1.1	0.3
Bladder/Serum	2.7	1.1	1.6	2.1	8.92	21.3	14.1	7.1	1.2	1.2	2.2	1.0
Prostate/Serum	0.74	0.5	0.9	1.4	1.31	5.8	3.2	1.4	0.17	1.0	0.9	6.0

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The decreased relative affinity of CU compared to D-CDU in the normal brain suggests that CU may not be optimal for targeting brain tumors using BNCT. The slower elimination of CU from prostate tumor xenografts compared to normal tissues, resulted in the greatest tumor/bladder and tumor/rectum ratios compared to D-CDU and D-ribo-CU, between 1 and 3 h following i.p. dosing. Therefore, CU should undergo further development for treating prostate cancer.

D-CDU may have theoretical advantages over *p*-boranophenylalanine (BPA) and borocaptate sodium (BSH), which are currently undergoing clinical testing in Japan and preclinical evaluation in the US for the treatment of brain tumors.[2–6,28,29] Unlike BPA which contains only one boron atom per molecule, D-CDU contains a carborane ring which delivers ten boron atoms per molecule accumulated in the tumor. Although BSH contains a dodecaborate cage moiety with two negative charges, which makes it extremely water soluble, the double negative charge may limit the ability of BSH to penetrate the cell membrane easily, resulting in low intracellular levels.[30–34] Due to the high LET α-particle released in BNCT, boron compounds that localized intracellularly near the nucleus have greater relative efficacy.[31]

The dose administered (5 mg/kg) was used to study the overall disposition of these agents in vivo. Previous studies with D-CDU have indicated that the compound has linear pharmacokinetics and is non-toxic in the range of 5 to 150 mg/kg.[15,26] Therefore, the dose could be increased for future efficacy studies of these agents to achieve higher tumor concentrations. The minimum effective dose of CU may be lower than predicted based on conventional BNCT dosimetry with boric acid (< 5 to 30 μg/g), which assumes a uniform distribution of ^{10}B throughout the cell.[28,30] D-CDU has previously shown efficacy against brain tumor isografts at concentrations of ^{10}B considered too low for therapeutic benefit (414.8 ng/g). The lipophilic agents related to D-CDU are more complex than boric acid. In addition, D-CDU and D-ribo-CU may be phosphorylated to produce anionic intracellular metabolites. Furthermore, the carboranyl moiety may be reduced to the anionic *nido* form. Localization or complexation of these metabolites with subcellular organelles and/or macromolecules may produce a non-uniform concentration in the cellular microenvirionments. Since the actual dosimetry of BNCT is related to the precise subcellular location of ^{10}B relative to the DNA, it is possible that cell lethality could result during BNCT at lower overall cellular concentrations.

This work lays the foundation for conducting BNCT studies in animal models of prostate cancer and represent the first publication on this important topic. We have discovered that high levels of CU accumulate in prostate tissue in mice. Theoretically, CU could be injected directly into prostate cancer tissues, since a significant retention of the compound is achieved in an animal model compared to normal tissue. Future efficacy studies are warranted to test the potential of this compound for the treatment of prostate tumors using BNCT. This study also demonstrates the importance of comparing the tissue dispositions of different nucleoside analogues and bases prior to the selection of a particular agent to target specific tumors for eventual treatment with BNCT.

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