FINAL PROGRESS REPORT

Reporting period: February 1, 2003 to July 31, 2003

Project title: Boron neutron capture therapy of brain tumors: targeting strategies and therapeutic models.

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DISCLAIMER

I. PROGRESS REPORT

Introduction

The gene encoding the epidermal growth factor receptor (EGFR) and its mutant isoform, EGFRvIII, frequently are overexpressed in malignant gliomas, and both are low or undetectable in normal brain. The overall goal of this project is to evaluate either boronated EGF or anti-EGFR monoclonal antibodies (MoAbs) as delivery agents for boron neutron capture therapy (BNCT). Using the F98 rat glioma model ($F98_{EGFR}$) cells, we previously have reported enhanced survival of $F98_{EGFR}$ glioma bearing rats following direct intratumoral (i.t.) injection of a boronated dendrimer (BD)-epidermal growth factor bioconjugate (BD-EGF), either alone or in combination with boronophenylalanine (BPA). These studies were the first *in vivo* data to establish *proof of principle* that a significant therapeutic gain could be obtained using a high molecular weight boron delivery agent. In order to increase the tumor uptake of BD-EGF, as previously reported, we have employed convection enhanced delivery (CED) to improve the targeting of $F98_{EGFR}$ gliomas. CED can increase intracerebral (i.c.) delivery of both high and low molecular weight agents to brain tumors by providing a pressure gradient to establish bulk flow during interstitial infusion in order to increase the volume of distribution. Based on these studies, which were carried out prior to funding of the present project, we have proceeded to investigate the following during the -01 project year.

1. Site-specific conjugation of boron-containing dendrimers to anti-EGF receptor monoclonal antibody cetuximab (IMC-C225) and its evaluation as a potential delivery agent for neutron capture therapy.

The purpose of this study was to investigate the use of the chimeric monoclonal antibody (MoAb) cetuximab (IMC-C225), which is directed against human wildtype EGFR and EGFRvIII, as a boron delivery agent for neutron capture therapy (NCT) of brain tumors. As determined by ^{125}I-cetuximab radioligand binding assays, F98 rat glioma cells, which had been transfected with the gene encoding EGFR ($F98_{EGFR}$), expressed $1.60\pm0.13\times10^5$ receptor sites/cell with a $K_a=1.64\pm0.32\times10^8$ M^{-1}. F98 cells transfected with the gene encoding a mutant form of EGFR, designated the $F98_{EGFRvIII}$ glioma, expressed $1.07\pm0.10\times10^5$ receptor sites/cell with a $K_a=2.18\pm0.54\times10^9$ M^{-1} compared to background levels expressed on F98 wild type cells ($F98_{WT}$). A heavily boronated, 5th generation polyamidoamine (PAMAM or "starburst") dendrimer, $G5\text{-}B_{1100}$, was linked to oligosaccharide moieties, which were distant from antigen binding sites of cetuximab, by means of the heterobifunctional reagents *N*-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) and *N*-(k-maleimidoundecanoic acid) hydrazide (KMUH). The resulting bioconjugate, designated $C225\text{-}G5\text{-}B_{1100}$, was separated from the unconjugated dendrimer using a Sephacryl S-300 column. Based on the relative concentration ratios of boron and protein, there were ~1100 boron atoms per molecule of cetuximab with only a slight reduction of K_a. The localization of $C225\text{-}G5\text{-}B_{1100}$ or $G5\text{-}B_{1100}$ in rats bearing intracerebral implants of either $F98_{EGFR}$ or $F98_{WT}$ gliomas was determined 24 h following direct intratumoral (i.t.) injection at which time 92.3±23.3 μg B/g tumor was localized in $F98_{EGFR}$ gliomas *versus* 36.5±18.8 μg B/g tumor in $F98_{WT}$ gliomas and 13.4±6.1 μg in normal brain. In contrast, only 6.7±3.6 μg B/g tumor of $G5\text{-}B_{1100}$ was localized in $F98_{EGFR}$ gliomas following i.t. injection, thereby demonstrating specific molecular targeting of EGFR. Based on these data, BNCT studies were initiated in $F98_{EGFR}$ glioma bearing rats to evaluate $C225\text{-}G5\text{-}B_{1100}$ for the treatment of intracerebral brain tumors.

2. Convection enhanced delivery of boronated bioconjugates to EGFR positive gliomas for BNCT

Convection enhanced delivery (CED) potentially is a powerful method to improve the targeting of low and high molecular weight agents to the central nervous system by applying a pressure gradient to establish bulk flow through the brain interstitium during infusion. The purpose of this study was to evaluate CED as a means to improve the i.c. and i.t. uptake of a heavily boronated macromolecule (BD) linked to either EGF or an anti-EGFRvIII MoAb L8A4, for NCT of rats bearing a syngeneic EGFR (+) glioma. The BD was linked to either EGF or L8A4 using heterobifunctional reagents. BD-EGF and BD-

L8A4 were radiolabeled with ^{125}I and administered by CED at a rate of 0.33 μl/min for 15, 30 and 60 min with corresponding volumes of infusion [V_i] of 5, 10 and 20 μl, respectively. The bioconjugates were administered by a syringe pump connected to an indwelling cannula implanted into the right caudate nucleus of non-tumor bearing rats or i.t. in rats bearing either $F98_{EGFR}$, $F98_{EGFRvIII}$ or $F98_{WT}$ gliomas. Animals were euthanized at 0, 6, 12 and 24 h after infusion and their brains were removed and serially sectioned at 2 mm intervals. The uptake and biodistribution of ^{125}I-BD-bioconjugates in tumor or normal tissues were studied by means of quantitative autoradiography (QAR) and γ-scintillation counting. The volume of distribution (V_d) in brain was assessed using a computer interfaced image analysis system. Following CED, the V_d increased from 34.4 to 123.5 μl with corresponding V_i ranging from 5 to 20 μl. The V_d of BD-EGF and BD-L8A4 in the brain was 64.8 μl and 59.8 μl, respectively, with CED (V_i 10 μ) and the V_d:V_i ratio was 6.1-7.0 compared to a V_d of 9.4-11.2 μl and a V_d:V_i ratio of 0.9-1.2 after direct i.c. injection. As determined by QAR and γ-scintillation counting at 24 h following CED, 47.4% of BD-EGF and 60.1% of BD-L8A4 were localized in $F98_{EGFR}$ and $F98_{EGFRvIII}$ gliomas compared to 33.2% of ID/g and 43.7% after direct i.t. injection and 12.3-15.2% ID/g in $F98_{WT}$ gliomas. Based on these observations, we have concluded that CED is more effective than i.t. injection as a way to deliver boronated EGF and MoAbs directed against EGFR or EGFRvIII (+)gliomas for neutron capture therapy and have employed CED in the studies described below.

II. FUTURE PLANS

Support from the Department of Energy for this project is gratefully acknowledged. This work is now being funded by National Institutes of Health grant # 1-R01 CA 098945-01A1 for a four year period that began June 1, 2003.

III. PUBLICATIONS *(Full length)*

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ABSTRACTS

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