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Evaluation of Boronated EGF as a Potential Delivery Agent for BNCT of Brain Tumors

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Introduction

The **epidermal growth factor receptor** (EGFR) gene is often amplified in human glioblastomas, but, reflecting the cellular heterogeneity of these tumors, the frequency of amplification is variable [1]. Since the number of EGFR on individual tumor cells may be 100 times greater than that on normal, untransformed cells [2], the EGFR has been considered as a potential target for the specific delivery of diagnostic and therapeutic agents to brain tumors. Initially, the focus was on using anti-EGFR monoclonal antibodies or their fragments [3], but within the past few years there has been increasing interest in using EGF based bioconjugates as targeting agents [4]. Recently, we have described a method for the boronation of EGF and have characterized the resulting bioconjugates *in vitro* [5]. In the present study,we have investigated the potential usefulness of **boronated EGF** as a delivery agent for neutron capture therapy in rats bearing intracerebral implants of the C6 glioma, which had been transfected with the gene encoding EGFR [6]. Our results indicate that following **intratumoral injection**, boronated EGF selectively targeted the transfected EGFR positive **C6 glioma**, and that the amount of delivered to the tumor exceeded by 3- 4 orders of magnitude that which could be delivered by intravenous injection.

Materials and Methods

Preparation of boronated EGF conjugates. "Starburst" dendrimers (SD), which are composed of repetitive polymido amino groups arranged in a starburst pattern, were boronated with a methylisocyanato polyhedral borane anion ($Na(CH_3)_3NB_{10}H_8NCO$), using a procedure recently described in detail by us [5]. Briefly summarized, EGF was first derivatized with the heterobifunctional reagent m-maleimidobenzoyl-N-hydroxysulfosuccinimide ester and then linked to boronated starburst dendrimer (BSD), which had been reacted with N-succinimidyl 3-(2-pyridyldithio) propionate.

$C6_{EGFR}$ cell line and in vitro studies. Rat C6 glioma cells were transfected with the gene encoding EGFR, and as determined by a radioligand binding assay and Scatchard analysis, the transfected cells, designated $C6_{EGFR}$ expressed 3 x10° EGFR per cells [6]. Receptor binding activity of boronated and native EGF with $C6_{EGFR}$ cells *in vitro* was studied by means of a competitive binding assay [5]. Boronated starburst dendrimer (BSD) and the EGF-BSD bioconjugate were iodinated with ^{131}I-NaI by means of the Bolton- Hunter reagent [7].

Animal model and in vivo biodistribution studies. C6 wildtype or $C6_{EGFR}$ glioma cells (10^4) were stereotactically implanted into the caudate nucleus of the right cerebral hemisphere of Fischer rats using a previously described procedure [6]. Four weeks following implantation, rats were divided into 4 experimental groups consisting of 15-18 animals each. Groups 1-3 had $C6_{EGFR}$ tumors and

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group 4 had C6 wildtype tumors. Groups 1 and 4 received an intratumoral (i.t.) injection of ^{131}I-labeled EGF-BSD (2 μci/ 4 μg EGF); group 2 received an intravenous (i.v.) injection of ^{131}I-labeled EGF-BSD; and group 3 received i.t. ^{131}I-labeled BSD. The biodistribution of ^{131}I-EGF-BSD and ^{131}I-BSD was studied by euthanizing the animals at 1, 6, 24 and 48 hours after injection. and then determining tissue and organ uptake by means of γ-scintillation counting using a well counter. In addition. some rats were studied by means of external γ-scintigraphy using a Technicare 438 gamma camera. Brains of those animals euthanized at 24 hours following intratumoral injection of ^{131}I-EGF-BSD were processed for autoradiography using stripping film with a one week exposure time. For microautoradiography, the sections were coated with NTB2 dipping emulsion (Kodak, Rochester. NY), and stored at 4^0 C in light-proof boxes for 3 weeks following which they were developed with Kodak D19 developer. Boron concentrations in tumor and normal tissues were determined by means of direct current plasma-atomic emission spectroscopy(DCP-AES) [8].

Results

Biodistribution. The biodistribution profiles of ^{131}I-EGF-BSD following i.v or i.t . injection into rats bearing $C6_{EGFR}$ gliomas are shown in Fig 1.

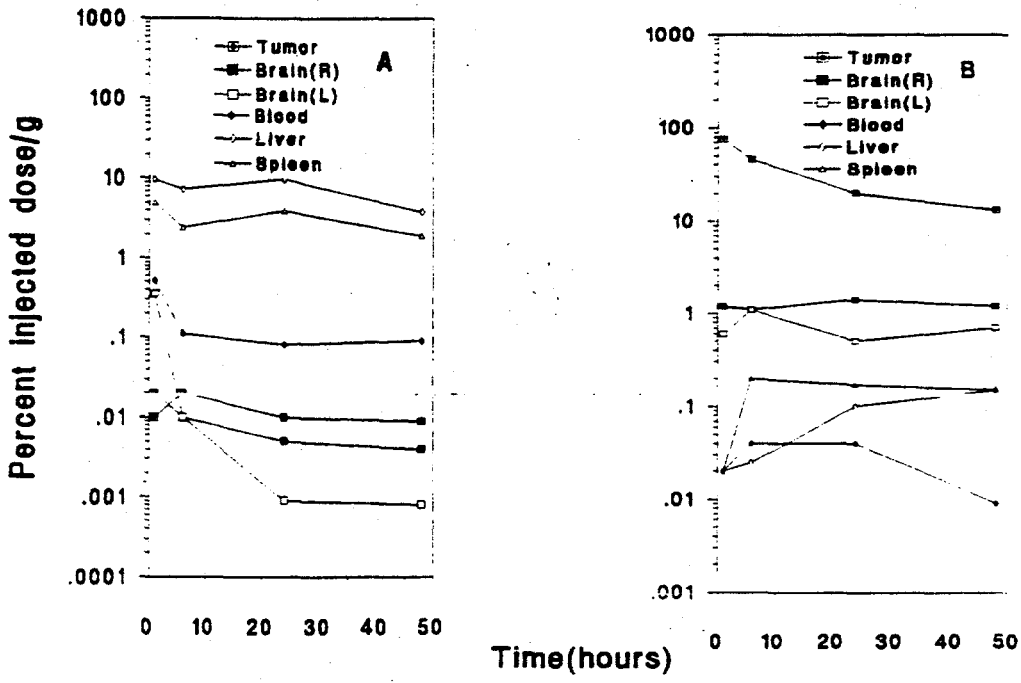

Fig.1 The biodistribution of ^{131}I-EGF-BSD following i.v.(A) or i.t.(B) injection into $C6_{EGFR}$ glioma bearing rats

Following i.t. injection of ^{131}I-EGF-BSD (Fig 1B). 21.8% of the injected dose per gram tissue (% ID/g) was localized in $C6_{EGFR}$ tumors at 24 h and 16.3% at 48h compared to 0.01% and 0.006% ID/g, respectively, for i.v.injected animals (Fig. 1A) . In contrast. following i.t. injection of EGF-BSD only 0.01-0.1% ID/g was localized in the liver and spleen at 24 and 48 h compared to 5-12% ID/g following i.v.injection. Tumor uptake of radioactivity following i.t. injection of ^{131}I-BSD or ^{131}I- EGF-BSD in rats bearing C6 or $C6_{EGFR}$ glioma are summarized in Fig 2. Between 1 and 6 hours following i.t. injection. 40-70% ID/g of EGF-BSD was non-specifically localized in $C6_{EGFR}$ and C6

gliomas, and the differences between the two groups were not statistically significant. By 24 h post injection, however, the amount of EGF-BSD in C6 wildtype, gliomas which do not express EGFR, had declined to 5% ID/g compared to 21.8%ID/g in $C6_{EGFR}$ gliomas Enhanced tumor uptake and persistence of the EGF-BSD bioconjugate in $C6_{EGFR}$ tumors was specifically determined by the EGF molecule, since only 3.4% ID/g of ^{131}I-BSD was detected in $C6_{EGFR}$ gliomas.

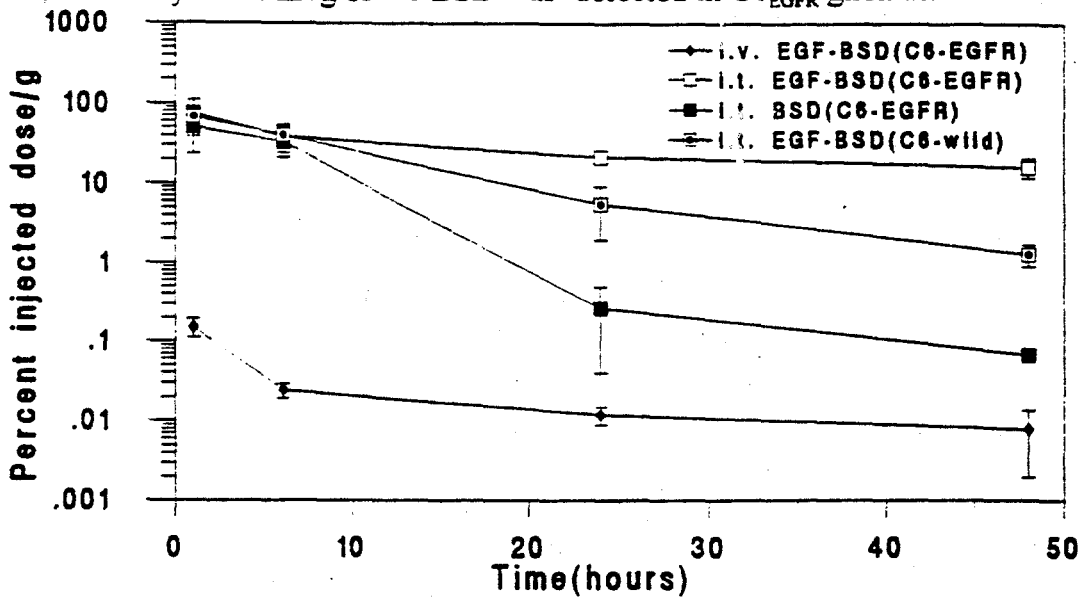

Fig. 2. Tumor uptake of radioactivity following i.t. injection of ^{131}I-EGF-BSD or ^{131}I-BSD in $C6_{EGFR}$ or C6 glioma bearing rats.

Autoradiography and external scintigraphy. Autoradiographs were made from brains of $C6_{EGFR}$ and C6 wildtype glioma bearing rats that has been euthanized at 24 h after i.t. injection of ^{131}I-EGF-BSD. Macro- and micro-autoradiograph showed that radioactivity had accumulated in $C6_{EGFR}$ tumors and brain surrounding tumor while, in contrast, there was no evidence of accumulation in C6 wildtype tumors. External γ-scintigraphy was carried out in tumor bearing rats at 24 and 48 h after i.t. administration of EGF-BSD. Tumors could be clearly visualized in $C6_{EGFR}$ glioma bearing rats, while in contrast, no tumors were evident in animals bearing C6 wildtype tumors. Following i.t. injection of EGF-BSD containing 34 μg of boron into $C6_{EGFR}$ tumors, tumor boron concentration was 15.2.7±5.2 μg/g (44.7% ID/g) at 24 hours, while boron concentrations in nomal brain, blood, liver kidney and spleen were all non-detectable(i.e. <0.5 μg/g).

Discussion

In the present study we have shown that high tumor uptake of BSD-EGF (21.8% and 16.5% ID/g at 24 h and 48 h, respectively) and low hepatic uptake(0.01-0.1%) were attained after i.t. injection of EGF-BSD into $C6_{EGFR}$ gliomas compared to low uptake in C6 wildtype tumors. In contrast, i.v administration of EGF-BSD resulted in low tumor uptake (0.01%) and high hepatic and splenic uptake (5-12%). In a recent phase I study to evaluate the possible use of a monoclonal antibody directed against EGFR to target human gliomas, the amount of antibody that reached the tumor following i.v. injection was in the range of 0.001 to 0.0001% ID/g [3]. These observations strongly suggest that it is highly unlikely that i.v. injection alone will be useful for delivering high molecular weight (HMW) agents to brain tumors and that other approaches will be required. Our data

demonstrated the striking advantage of i.t versus i.v injection for a HMW agent. Intratumoral injection of EGF has circumvented the most serious problems asociated with systemic delivery of EGF bioconjugate, low tumor and high hepatic uptake. External γ-imaging and autoradiography demonstrated that radiolabed EGF concentrated in $C6_{EGFR}$ but not C6 wildtype gliomas at 24 h and 48 h folowing i.t. injection of ^{131}I-EGF-BSD, indicating that tumor uptake and retention were EGFR dependent. The high hepatic uptake of EGF-BSD, in part, may be related to the propensity of both BSD and EGF to localize in the liver. Our previous studies have shown that SD localized in liver and spleen, and that the amount appeared to be directly related to the molecular weight and number of reactive terminal amino groups [9]. Olsson et al. (these proceedings) have reported that hepatic and renal uptake of ^{125}I-EGF-dextran-BSH [10] was high following i.v. administration and tumor uptake was very low. We recently have initiated studies on the biodistribution of ^{99m}Tc-EGF in rats bearing $C6_{EGFR}$ gliomas following i.v or i.c. administration and have observed by means of external γ-scintigraphy that EGF had a high propensity to localize in liver, spleen and kidneys. This observation further supports our view that i.t. administration may be the most effective route for the delivery of either boronated or radiolabeled EGF to brain tumors, and that i.v. administration would not result in sufficient amounts of either boron-10 or radionuclide for effective therapy. Studies currently are in progress to optimize i.t. delivery and to assess its efficacy for BNCT of EGFR positive rat brain tumors.

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