Published in final edited form as:

J Control Release. 2011 November 7; 155(3): 393–399. doi:10.1016/j.jconrel.2011.06.033.

Brain Tumor Targeting of Magnetic Nanoparticles for Potential Drug Delivery: Effect of Administration Route and Magnetic Field Topography

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Abstract

Our previous studies demonstrated feasibility of magnetically-mediated retention of iron-oxide nanoparticles in brain tumors after intravascular administration. The purpose of this study was to elucidate strategies for further improvement of this promising approach. In particular, we explored administration of the nanoparticles via a non-occluded carotid artery as a way to increase the passive exposure of tumor vasculature to nanoparticles for subsequent magnetic entrapment. However, aggregation of nanoparticles in the afferent vasculature interfered with tumor targeting. The magnetic setup employed in our experiments was found to generate a relatively uniform magnetic flux density over a broad range, exposing the region of the afferent vasculature to high magnetic force. To overcome this problem, the magnetic setup was modified with a 9-mm diameter cylindrical NdFeB magnet to exhibit steeper magnetic field topography. Six-fold reduction of the magnetic force at the injection site, achieved with this modification, alleviated the aggregation problem under the conditions of intact carotid blood flow. Using this setup, carotid administration was found to present 1.8-fold increase in nanoparticle accumulation in glioma compared to the intravenous route at 350 mT. This increase was found to be in reasonable agreement with the theoretically estimated 1.9-fold advantage of carotid administration, R_d . The developed approach is expected to present an even greater advantage when applied to drug-loaded nanoparticles exhibiting higher values of R_d .

Keywords

iron oxide nanoparticles; magnetic targeting; brain tumor targeting; intra-carotid administration; magnetic field topography

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1. Introduction

Malignant brain tumors are one of the most lethal forms of cancer. Median survival for patients with malignant glioma is only 48 weeks from diagnosis [1, 2]. Current treatment modalities, including surgery, radiotherapy and chemotherapy, fail to improve this dismal prognosis [1, 2]. Although many potent chemotherapeutic agents are now available, their delivery to the tumor site after systemic administration faces many difficult challenges, resulting in subtherapeutic exposure of the tumor tissue to the drug [3]. To enhance drug exposure, use of colloidal drug carriers (e.g. liposomes, nanoparticles) has been extensively explored [4, 5]. Accumulation and retention of a drug-loaded carrier at the brain tumor site could allow localized release of a drug, thereby enhancing tumor exposure to the cytotoxic agent.

Iron-oxide based nanoparticles present a particularly interesting platform for drug delivery due to their magnetic properties [6, 7]. Magnetic responsiveness of the iron oxide core allows utilization of these nanoparticles for magnetic targeting. Magnetic targeting has recently emerged as a promising approach for localized retention of drug-loaded nanocarriers in tumor lesions [6, 7]. Studies in animal tumor models demonstrated that drug-loaded magnetic nanoparticles, injected directly into arterial blood supply of subcutaneous tumors, could be locally retained within the tumor tissue by an externally applied magnetic field [8, 9].

In contrast to readily accessible subcutaneous tumor lesions, brain tumors present a more challenging situation. Their deep intracranial location precludes direct administration of the nanoparticles into local vasculature of the tumor without direct brain intervention. Nevertheless, our recent studies in 9L-glioma bearing rats demonstrated that magnetic targeting of brain tumors following intravenous administration is a feasible approach [10]. These studies revealed that intravascularly administered nanoparticles are passively delivered to the brain tumor vasculature even in the absence of magnetic field. However, magnetic targeting conferred prolonged retention of the nanoparticles within glioma lesions, resulting in a 5-fold increase in nanoparticle exposure of the tumor compared with non-targeted animals [10]. Further improvement of this promising strategy requires the understanding and optimization of factors affecting both the passive presentation of nanoparticles to tumor capillary bed and the magnetic retention.

One important factor affecting the exposure of tumor vasculature to nanoparticles is the route of their administration. Intra-arterial administration of nanoparticles was shown to present an advantage in previous magnetic targeting studies with rats bearing subcutaneous tumors [9]. The main potential advantage of intra-arterial administration over the intravenous route is that the vasculature of the tissue perfused by the injected artery receives higher plasma concentration during the first passage through the circulation [3]. Mathematical analysis indicated that the intra-arterial advantage is highest under conditions of low blood flow rate in the infused artery and high systemic clearance of the agent [11, 12]. Carotid artery administration was explored for chemotherapeutic agent delivery to brain tumors in preclinical and clinical settings and demonstrated to provide higher tumor exposure for rapidly eliminating drugs [13, 14]. Magnetic nanoparticles typically exhibit short plasma half-lives, and therefore are good candidates for intra-carotid administration [15]. With recent advances in endovascular technology and the increased safety of angiographic procedures, carotid administration of nanoparticles can offer a clinically-relevant alternative to intravenous administration [16].

However, achieving a high first-pass nanoparticle concentration with intra-carotid administration can also present a serious pitfall in conjunction with magnetic targeting.

Previous studies in mice, injected with high concentration of iron-oxide nanoparticles, demonstrated that tumor-supplying artery exposed to magnetic field became mechanically occluded due to nanoparticle aggregation [17]. Occlusion of the carotid artery, which directly supplies the normal brain parenchyma, can lead to severe neurological and neurocognitive sequelae [18] and must be avoided. Magnetic field topography and strength have been shown to play a central role in modulating the extent of nanoparticle aggregation at arterial and capillary blood flow rates [19]. Therefore, characteristics of the magnetic field source need to be carefully adjusted to achieve desirable tumor retention of nanoparticles while avoiding undesirable arterial agglomeration with intra-carotid administration.

The goal of this study was to examine potential strategies for optimization of magnetically-mediated delivery of blood-born iron oxide nanoparticles to brain tumor lesions. In particular, we compared the effect of intracarotid versus intravenous administration of nanoparticles on their tumor accumulation in 9L glioma-bearing rats. In addition, we optimized magnet source characteristics including field topography, strength and gradient to improve the selectivity and extent of nanoparticle retention at the target site.

2. Materials and Methods

2.1 Iron oxide nanoparticles

Iron oxide nanoparticles coated with starch (G100) were generously contributed by Chemicell ® (Berlin, Germany). The physical properties of G100 were previously reported [10]; the particle size was measured to be 110 ± 22 nm, and the zeta-potential was -12 ± 6 mV. Iron concentration of nanoparticle preparations was determined by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-EOS) as previously described [10].

2.2 Modeling of magnetic field topography

Magnetic field topography was simulated using Magnetism 3D software package (University of California, San Diego, CA). Components chosen for simulation analysis were limited to those readily available for implementation in our laboratory and included a dipole electromagnet and a set of permanent magnets of variable geometry and strength. Models involving different modifications to a standard dipole electromagnet were constructed and the corresponding field topography simulated in a 3D mode. The parameters varied included the air gap between the poles of the electromagnet and strength, geometry and orientation of small permanent magnet components.

2.3 Measurements of magnetic flux density

Measurements of magnetic flux density were performed using a 3-axis Hall Teslameter (Metrolab THM 7025, GMW Associates, CA). The instrument simultaneously measures the three mutually perpendicular field components (B_x , B_y and B_z) and calculates the modulus of

the magnetic field ($B = \|\vec{B}\| = \sqrt{B_x^2 + B_y^2 + B_z^2}$) at the measured location. The energy density scalar field $\vec{B} \cdot \vec{B} = B_x^2 + B_y^2 + B_z^2$ could be then easily calculated as the square value of

magnetic field modulus at each measured point of the mapped space: $(\vec{B} \cdot \vec{B})_{x,y,z} = \|\vec{B}\|_{x,y,z}^2$

2.4 Magnetic field source

A standard dipole electromagnet (GMW Associates, Model 3470) was used as the magnetic field source in targeting experiments. To obtain a sharper magnetic field gradient within the target region, several modifications to the standard electromagnet configuration were assessed. Modeling of magnet configurations and simulations of the corresponding magnetic

field topographies were performed as described in section (2.2). To realize the model configuration generating a sharper field gradient, a small cylindrical neodymium-iron-boron magnet (NdFeB, 9 mm in diameter) was attached to the pole of the electromagnet.

2.5 In Vivo studies

All animal experiments were conducted according to protocols approved by the University of Michigan Committee on Use and Care of Animals (UCUCA).

2.5.1 Pharmacokinetic analysis—The pharmacokinetics of G100 magnetic nanoparticles were studied in male Fisher 344 rats weighting 200–250g as previously described [20]. Briefly, the nanoparticle suspension in PBS was administered to rats via the tail vein catheter at a dose of 12 mg Fe/kg body weight. Blood samples of 100 μ L were collected from the cannulated carotid artery in 0.5 ml Eppendorf tubes spiked with 10 μ L of Heparin solution (5,000 USP Units/ml). Samples were acquired before and serially after the nanoparticle administration at pre-set time intervals for 60 minutes. Plasma fractions were immediately separated by centrifugation (3 minutes at 7,000g) and stored at -80°C .

2.5.2 Induction of brain tumors—Intracerebral 9L tumors were induced in male Fisher 344 rats weighting 125–150 g according to a previously described procedure [21]. Briefly, rat 9L-glioma cells (Brain Tumor Research Center, University of California, San Francisco) were grown to confluence in 100 mm culture dishes. The harvested cells were resuspended in serum free DMEM and implanted ($\sim 10^6$ cells in 10 μ L) in the right forebrain of the animals at a depth of 3 mm beneath the skull through a 1-mm-diameter burr hole. The tumor volume was monitored with MRI beginning on day 10 after cell implantation to select tumors between 70 and 90 μ L for magnetic targeting experiments.

2.5.3 Magnetic resonance imaging—MRI experiments were performed on a 18-cm horizontal-bore, 7 Tesla Varian Unity Inova imaging system (Varian, Palo Alto, CA). Animals were anesthetized with 1.5% isoflurane/air mixture and imaged using a 35-mm-diameter quadrature RF head coil (USA Instruments Inc, OH). To visualize the tumor localization within the rat brain, 13 axial sections of the brain were acquired with a T_2 -weighted fast spin echo sequence using the following parameters: repetition time (TR) = 4 s, echo time (TE) = 60 ms, field of view = 30×30 over 128×128 matrix, slice thickness = 1mm, slice separation = 2 mm, four signal averages per phase encoding step. To determine nanoparticle distribution in the brain, 13 gradient echo (GE) axial slices of the brain were collected before the nanoparticle administration (baseline scans) and immediately following magnetic targeting. GE images were acquired with the following parameters: TR = 20 ms, TE = 5 ms, field of view = 30×30 over 128×128 matrix, slice thickness = 1 mm.

2.5.4 Magnetic targeting—Magnetic targeting studies were carried out in tumor-bearing rats using either intravenous or intra-arterial route for nanoparticle administration. For intravenous administration the tail vein was cannulated with a 26-gauge angiocatheter (AngiocathTM, Becton Dickinson, Sandy, UT). For intra-arterial administration, the right carotid artery was cannulated by a method that did not require vessel occlusion, as previously described [22]. Briefly, the catheter consisted of fused silica capillary tubing (Polymicro Technologies, Phoenix, AR) and polyethylene tubing (PE-10, Braintree Scientific inc., MA). The sharpened silica tubing was used to puncture the exposed arterial wall and inserted into the artery. The tiny incision was quickly resealed with a small drop of cyanoacrylate tissue adhesive (3M Vetbond), avoiding permanent artery occlusion and preserving intact blood flow through the vessel.

During experiments involving the standard magnet configuration, animals were placed supinely on the platform with their heads positioned in the airgap between the poles of the electromagnet. For experiments performed with the modified magnet configuration, the animal head was positioned directly on the pole face of the small magnet. In each case, the tumor lesion was located approximately 5 mm away from the magnetic pole face. The magnetic field density at the pole face of the small magnet was adjusted to 0, 150 or 350 mT. Animals were then injected, through either an intravenous or intracarotid catheter with nanoparticle suspension in PBS at a dose of 12 mg Fe/kg and retained in the magnetic field for 30 min. The rats were imaged with MRI before the administration of nanoparticles and after the magnetic targeting as described in Section 2.5.3. Immediately following MRI, the animals were sacrificed and dissected. The isolated brain was divided into right and left hemispheres and the tumor was carefully separated from the normal tissue of the right hemisphere. The left hemisphere and the tumor tissues were frozen and kept at -80°C .

2.6 *Ex vivo* analysis of tissue and plasma samples by Electron Spin Resonance (ESR) spectroscopy

Nanoparticle concentrations in plasma and tissue samples were determined as previously reported [23]. Briefly, ESR spectra of the samples were acquired using an EMX ESR spectrometer (Bruker Instruments Inc., Billerica, MA) equipped with a liquid nitrogen cryostat. The acquisition parameters were: resonant frequency: $\sim 9.2\text{GHz}$, microwave power: 20mW, temperature: 145K, modulation amplitude: 5G and receiver gain of 5×10^4 and 5×10^3 for tissue and plasma samples, respectively.

2.7 Statistical analysis

Data are presented as mean $\pm$ SE, unless indicated otherwise.

SPSS 15.0 statistical software package (SPSS Inc., Chicago, IL) was used for data analysis. The two-way ANOVA was used to analyze the significance of the flux density and the effects of administration route on nanoparticle accumulation in tumor or contra-lateral brain tissues. A general linear model including the main effects and the interaction term was employed. The effect of the administration site at each level of flux density was further evaluated for significance with a series of planned orthogonal contrasts. A p-value of <0.05 was considered statistically significant.

3. Results

3.1 Estimated advantage of intra-carotid nanoparticle administration

First, we wished to examine whether intra-carotid nanoparticle administration would provide an advantage in nanoparticle exposure of the tumor vasculature compared to the intravenous route. It has been mathematically derived that the advantage in delivery to the tumor site resulting from arterial infusion (R_d) is equal to the ratio of the areas under the arterial concentration-time curve (AUC) obtained after arterial and venous administration, respectively [12]. It was also shown that the value of R_d can be experimentally estimated from i.v. bolus injection data using the following expression [12]:

$$R_d = (I_a + I_{ra}) / (I_v + I_{rv}), \quad I_a = \frac{D}{F}, \quad I_v = \frac{D}{CO} \quad [1]$$

where, I_a and I_v are the arterial AUCs for the first passage of a substance through the arterial blood after arterial and venous administration, respectively. I_{ra} and I_{rv} are the arterial AUCs

for the systemic recirculation of the substance after arterial and venous administration, respectively.

D is the dose, F the blood flow through the infused artery, and CO the cardiac output.

To determine I_{rv} we measured nanoparticle concentrations in arterial plasma as a function of time after intravenous bolus injection. The obtained pharmacokinetic profile is presented in Figure 1. Since magnetic targeting was continued for 30 minutes after nanoparticle administration, the relevant value of I_{rv} was calculated on the 0–30 min interval of the concentration-time curve and found to be $934 \pm 44 \mu\text{g Fe/ml}\cdot\text{min}$. It was postulated that intra-arterially administered nanoparticles return to the systemic circulation after the first passage through the tumor vasculature and are distributed as though they were administered intravenously [12]. Assuming that the fraction of total dose lost during first passage through the tumor vasculature is small, the systemic recirculation AUC for intra-arterial administration I_{ra} is approximately equal to I_{rv} . With literature values for the carotid blood flow and cardiac output measured in Fisher rats (see Table 1), the expected advantage of intra-carotid administration, R_d , was estimated to be 1.9.

3.2 Magnetic targeting with intra-carotid administration of nanoparticles using the standard dipole electromagnet

The calculated R_d indicated that intra-arterial nanoparticle administration would confer a 1.9-fold higher exposure of tumor vasculature to magnetic nanoparticles as compared to the intravenous injection. This promising estimate prompted us to experimentally examine the effect of intra-arterial administration on nanoparticle capture at the tumor site. We first used the magnetic setup employed in our previous study [10]. The setup involved positioning of the rat head in the airgap between the poles of a standard dipole electromagnet. A typical series of MRI images acquired before and after intra-carotid nanoparticle administration in conjunction with the dipole magnetic setup are presented in Figure 2. The brain tumor lesion is clearly visible as a hyperintense region on the T₂-weighted baseline scan (Figure 2A). Iron oxide nanoparticles manifest themselves by pronounced hypointensity on the GE MRI images. While only a minor loss of signal is observed in the tumor region on the GE post-targeting scan, a very pronounced and broad hypointense area is apparent on the right side of the animal head (Figure 2C). This hypointense area reveals local aggregation of magnetized nanoparticles in the afferent vasculature.

3.3 Design of a magnetic setup with optimized magnetic field topography

To analyze the possible cause for nanoparticle aggregation after intra-carotid administration, we evaluated the topography of the magnetic field generated by the employed magnetic setup. The magnetic force responsible for retention of a superparamagnetic nanoparticle in a non-uniform magnetic field can be expressed by Equation 1 [26]:

$$\vec{F}_m = -\Delta\chi V_m \nabla u_m = \Delta\chi V_m \frac{\nabla B^2}{2\mu_0} \quad [1]$$

where $\nabla\chi$ is the difference in magnetic susceptibilities of the nanoparticles and the medium, V_m - the magnetic volume of nanoparticle, μ_0 - permeability of free space, B - magnetic field, and u_m - the magnetostatic energy density.

It is apparent from equation (1) that the magnetic force on given magnetizable particles suspended in a given medium is proportional to the gradient of the magnetostatic energy density:

$$\vec{F}_m \propto \frac{\nabla B^2}{2\mu_0} \quad [2]$$

To calculate ∇B^2 , we mapped the magnetic flux density in the area of the head and neck of the animal. The flux density magnitude was found to decay very slowly with longitudinal distance from the tumor (Figure 3: A, B – “original” setup). For example, while the measured flux density at the tumor location was 376 mT, the value recorded 2.5 cm caudal to the tumor was 218 mT, indicating that the afferent arterial vasculature would be exposed to relatively high magnetic fields. The 3D-mapping of the magnetic flux density allowed calculation of the magnitude and direction of the ∇B^2 vector at the tumor and the carotid injection sites (Figure 3C). The magnitude of ∇B^2 at the carotid site (2.0 T²/m) was found to be only 3.6-fold lower than that at the tumor location (7.4 T²/m). We hypothesized that the exposure of the neck region of the animal to high magnetic force could be responsible for nanoparticle aggregation in the artery.

We further hypothesized that modification of magnetic field topography to achieve reduction in ∇B^2 at the carotid injection site, while maintaining high ∇B^2 at the target tumor site might help to avoid aggregation of the nanoparticles in the afferent vasculature without compromising nanoparticle capture in the tumor. To address this hypothesis we first evaluated magnetic field topographies of several magnetic setup configurations using theoretical analysis (see Section 2.3). The goal of this analysis was to select a configuration which would generate a narrow region of high magnetic flux density and minimize the flux in the surrounding area. A promising configuration suggested by the modeling included the placement of a small cylindrical magnet (9 mm in diameter) on the tapered electromagnet pole. Models of the original and the optimized magnet configurations, with their corresponding flux density profiles, are presented in Figures 4A and 4B, respectively. The small cylindrical magnet appeared to serve as a low resistance path for diverting field lines from the electromagnet. Furthermore, as seen in Figures 4C and 4D, the magnetic flux maps measured for the two magnet configurations exhibited topography which was consistent with the theoretically predicted profiles. Homogeneous broad-range flux topography of the electromagnet (Figure 4C) was contrasted with the focused flux topography of the optimized configuration (Figure 4D). The latter configuration resulted in an increase of the magnetic flux at the face of the small magnet from 150 mT to 350 mT. More importantly, the flux was found to decrease much more rapidly with distance than seen with the original setup (Figure 3B). The magnitude of the ∇B^2 vector at the carotid injection site (Figure 3C) was reduced 6-fold with the modified magnet configuration (0.34 T²/m) compared with the original one (2.0 T²/m), when both setups were operated at the same input current of 4.8 A. In contrast, at the tumor site the magnitude of ∇B^2 was found to be 1.9-fold higher with the modified (14.4 T²/m) than with the original setup (7.4 T²/m).

3.4 In vivo magnetic targeting using the modified magnet configuration

Magnetic targeting experiments with carotid administration were repeated to assess the effect of the modified magnetic setup (Section 3.3). A subset of MRI images acquired before nanoparticle administration and after magnetic targeting in a representative rat is presented in Figure 5. The post-targeting GE image (Figure 5:3) demonstrates that the problem of non-specific aggregation in the afferent vasculature has been overcome. Moreover, the hypointense region on this image corresponds to the tumor location visible on the T₂-weighted baseline scan (Figure 5:1), indicating accumulation of the nanoparticles within the glioma lesion.

We next sought to compare the tumor accumulation of magnetic nanoparticles following intra-venous and intra-carotid administration routes using the modified magnet configuration. In addition, to evaluate the effect of the field strength, targeting experiments were performed with the tumor site flux densities adjusted to 0, 150 and 350 mT. The nanoparticle concentrations in tumor and contra-lateral normal brain were determined by EPR analysis and are presented in Figure 6. At the flux density of 150 mT, mean concentration of magnetic nanoparticles in tumor of intra-carotid injected rats (19.9 ± 8.9 nmol Fe/g tissue) was not significantly different ($p=0.13$) from that in the intravenously injected group (14.4 ± 4.5 nmol Fe/g tissue). In contrast, at the flux density of 350mT, nanoparticle accumulation in tumor lesion was found to be 1.8-fold higher ($p=0.025$) with carotid (42.7 ± 19.5 nmol Fe/g tissue) than with intravenous administration (24.3 ± 7.2 nmol Fe/g tissue). Interestingly, at the same flux density of 350 mT, nanoparticle accumulation in the contra-lateral normal brain (4.6 ± 2.9 vs. 6.3 ± 1.4 nmol Fe/g tissue for the carotid and intravenous administrations, respectively) was not found to be significantly affected ($p=0.17$) by the route of administration, indicating that the specificity of tumor targeting was not compromised by the intra-carotid administration.

4. Discussion

Magnetic targeting of iron oxide nanoparticles was previously shown to present a promising potential for delivery of drugs to brain tumors [10]. To further optimize this approach, it is essential to better understand the factors affecting nanoparticle capture at the brain tumor site. The process of magnetic targeting following intravascular administration can be conceptually divided into two consecutive components: a passive delivery of nanoparticles to the tumor vasculature and the magnetic entrapment of nanoparticles presented to tumor capillaries. The amount of agent passively delivered to the tumor over time is known to be proportional to the area under the concentration-time curve (AUC) of the agent in arterial blood [12]. Intra-arterial administration can increase arterial AUC due to the fact that the arterial blood is exposed to higher concentration of the agent on the first passage through circulation. To this regard, we sought to examine whether intra-carotid administration would present an advantage over the intravenous route with magnetic targeting. The theoretical estimate of intra-arterial advantage R_d , determined to be 1.9 for iron oxide nanoparticles, suggested a nearly doubled exposure of tumor vasculature with intra-carotid versus intravenous administration.

However, our first attempt to target iron oxide nanoparticles with intra-carotid administration failed due to aggregation of nanoparticles in the afferent vasculature. Previous work has shown that magnetic targeting of nanoparticles in the bloodstream relies on the interplay between the magnetic force and the hydrodynamic drag [26]. The magnetic capture of nanoparticles occurs only when the magnetic force is greater than the opposing drag force. To maintain physiologically relevant hydrodynamic drag, it is essential to preserve the intact flow through carotid artery during targeting experiments. Yet, carotid catheterization procedures that are commonly performed in rodents are carried out with permanent ligation of the carotid artery, involving complete blockage of the flow [27]. In contrast, the procedure used in this study did not require vessel occlusion for catheter placement, thus preserving intact hydrodynamic component in the afferent vasculature.

The occurrence of arterial nanoparticle aggregation even in the presence of an intact hydrodynamic force suggested that aggregation was a result of the magnetic component of the targeting procedure. Examination of the magnetic field produced by the standard electromagnet showed a relatively uniform magnetic flux density which decayed slowly with distance and exposed the region of the afferent vasculature to high magnetic force. We sought to reduce the magnetic force at the carotid injection site in order to allow the drag to

sweep the nanoparticles downstream with the bulk flow. Our modified magnetic setup exhibited steeper magnetic field topography, resulting in 6-fold reduction of the magnetic force at the injection site. This force reduction was found sufficient to alleviate the arterial aggregation problem under the conditions of intact carotid blood flow. Elimination of the non-specific nanoparticle aggregation in the afferent vasculature allowed us to pursue magnetic targeting experiments using carotid administration.

We then proceeded to compare the intra-carotid and intravenous routes of administration while subjecting the tumor site to two different magnetic flux densities. Our results revealed that the magnetic flux density at the target site dictated whether carotid administration would result in an advantage in nanoparticle accumulation within tumor lesion. While no significant difference was observed between the carotid and intravenous administration routes with the flux density of 150 mT, intra-carotid administration resulted in 1.8-fold higher nanoparticle accumulation in the tumor with the higher flux density of 350 mT. The advantage of carotid administration with respect to the nanoparticle exposure is gained only on the first passage through the tumor vasculature [12]. After the first passage, nanoparticles enter the systemic blood pool and circulate as if they were administered intravenously. Accordingly, our results indicated that the magnetic force present at a flux density of 150 mT was not sufficient to achieve significant nanoparticle retention on the first pass after carotid administration. Tumor entrapment of the recirculated nanoparticles was therefore similar to that achieved with intravenous administration. In contrast, the flux density of 350 mT generated a sufficiently high magnetic force to entrap a fraction of nanoparticles on their initial passage through the tumor vasculature. Interestingly, the increased tumor nanoparticle accumulation after intra-carotid administration was not accompanied by a respective increase in nanoparticle concentration in the contra-lateral brain compared to intravenous administration. Thus, carotid administration also presents a way to improve tumor selectivity of nanoparticle delivery.

The 1.8-fold increase in tumor accumulation of nanoparticles with carotid versus intravenous administration at 350 mT is in reasonable agreement with the theoretical estimated R_d of 1.9. This agreement indicates that the potential of carotid administration, due to higher exposure of tumor vasculature to nanoparticles, has essentially been realized for nanoparticles employed in the present study. Nevertheless, our approach can be foreseen to present much greater advantage in systems exhibiting higher values of R_d . The value of R_d depends on nanoparticle pharmacokinetics [12]. In this study carotid and intravenous routes of administration were compared using nanoparticles with slightly negative surface charge (ζ -potential = -12 ± 6 mV). These nanoparticles, devoid of payload, exhibited a plasma half-life sufficient for their presentation at the tumor site, thus reducing the advantage of carotid administration. However, complexation of nanoparticles with drugs has been shown to alter both the nanoparticle surface charge and their pharmacokinetic behavior. For example, doxorubicin loading was demonstrated to confer nanoparticle surface with a positive charge, thus significantly reducing nanoparticle circulation half-life and compromising their passive delivery to brain tumor lesions after intravenous administration [28]. Under such conditions, carotid administration might present a greater advantage over the intravenous route than that seen with the non-loaded nanoparticles in the present study.

In summary, the strategies of carotid administration and selective magnetic entrapment examined here contribute to the understanding of factors affecting magnetic targeting of brain tumor lesions. A simple modification of magnetic field topography was designed and implemented to successfully avoid the aggregation of nanoparticles in the afferent vasculature. Using this setup, carotid administration was evaluated versus the intravenous route and found to present a 1.8-folds advantage with respect to nanoparticle accumulation

in glioma with an applied magnetic field of 350 mT, but not with 150 mT. These results revealed that strong magnetic interaction at the tumor site is required to gain an advantage with the carotid administration route. We expect that the developed approach will provide even greater advantage when applied to drug-loaded nanoparticles exhibiting shorter plasma half-lives.

Acknowledgments

We thank Dr. Christian Bergemann from Chemicell (Germany) for the generous gift of iron oxide nanoparticles. We acknowledge the Center for Molecular Imaging at the University of Michigan for providing access to the MRI facility. This work was supported in part by NIH RO1 Grants CA114612 and NS066945, as well as a research grant from the Hartwell Foundation. This research was also partially sponsored by Grant R31-2008-000-10103-01 from the World Class University (WCU) project of the MEST and NRF of South Korea. Victor C. Yang is currently a Participating Faculty in the Department of Molecular Medicine and Biopharmaceutical Sciences, College of Medicine & College of Pharmacy, Seoul National University, South Korea. In addition, the project was partially sponsored by the National Basic Research Program of China (973 Program) 2007CB935800. Beata Chertok was a recipient of the University of Michigan Rackham Graduate School Pre-doctoral Fellowship.

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Figure 1. Concentration-time profile for G100 in rat plasma following i.v. administration of the nanoparticles at a dose of 12 mg Fe/kg. Data presented as Mean $\pm$ SD, n=4

**T₂,
baseline**

**GE,
baseline**

**GE,
30 min**

Figure 2.

Typical set of axial MRI scans of the rat head acquired before carotid injection of magnetic nanoparticles (baseline) and 30 minutes after magnetic targeting with the original dipole magnetic setup.

Figure 3.

A. Schematic depiction of the coordinate system with respect to the animal body used for magnetic flux density mapping at the tumor (T) and the carotid injection site (C). B. Magnetic flux density profile measured along the dashed line (a in A) in Y direction with the original and the modified magnetic configurations. C. Vectors of the magnetostatic energy density gradient (∇B^2) at the tumor and the carotid injection sites calculated for the original and the modified magnetic configurations. The coordinate systems for the tumor and the carotid site correspond to those schematically depicted in (A).

Figure 4. Magnetic flux topography of the optimized and the standard magnet configurations. (A) and (B) display models of the original and the modified magnetic configurations, respectively, with their corresponding magnetic flux density profiles as calculated using Magnetism 3D software package. The dashed lines indicate the plane on which the head of the tumor-bearing animal was placed. (C) and (D) display magnetic flux maps measured with Hall Teslameter for the original and the modified magnet configurations, respectively, along the targeting plane.

**T₂,
baseline**

**GE,
baseline**

**GE,
30 min**

Figure 5.

Typical set of axial MRI scans of the rat head acquired before carotid injection of magnetic nanoparticles (baseline) and 30 minutes after magnetic targeting with the modified magnetic configuration.

Figure 6.

Analysis of nanoparticle concentrations in excised tumor and contra-lateral brain tissues of magnetically targeted rats following intra-carotid (carotid) and intravenous (iv) administration. The magnetic targeting was carried out with a modified magnetic setup at different magnetic flux densities.

Table 1Parameters used for estimation of the advantage of intra-carotid nanoparticle administration, R_d

Parameter	Symbol	Units	Value	Reference
Carotid blood flow	F	ml/min	2.63 ± 0.14	[24]
Cardiac output	CO	ml/min	106 ± 5	[25]
Dose	D	μgFe	2400	Present study
Arterial recirculation AUC following iv injection	I_{rv}	$\mu\text{gFe/ml*min}$	934 ± 44	Present study