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Transient cerebral hypoperfusion assisted intraarterial cationic liposome delivery to brain tissue

Shailendra Joshi, MD^a, Rajinder P. Singh-Moon, BS^a, Mei Wang, MPH^a, Durba B. Chaudhuri, MS^b, Mark Holcomb, PhD^d, Ninfa L. Straubinger, BS^f, Jeffrey N. Bruce, MD, FACS^e, Irving J. Bigio, PhD^{b,c}, and Robert M. Straubinger, PhD^{f,g}

^aDepartment of Anesthesiology, College of Physicians and Surgeons of Columbia University, New York, NY 10032

^bDepartment of Electrical Engineering, Boston University, Boston, MA 02215

^cDepartment of Biomedical Engineering, Boston University, Boston, MA 02215

^dDepartment of Physics, School of Art and Science, Susquehanna University, Selinsgrove, PA 17870

^eDepartment of Neurosurgery, College of Physicians and Surgeons of Columbia University, New York, NY 10032

^fDepartment of Pharmaceutical Sciences, University of Buffalo, State University of New York, Buffalo, NY 14214

^gDepartment of Pharmacology & Therapeutics, Roswell Park Cancer Institute, Buffalo, NY 14263

Abstract

Object—Transient cerebral hypoperfusion (TCH) has empirically been used to assist intraarterial (IA) drug delivery to brain tumors. Transient (< 3 min) reduction of cerebral blood flow (CBF) occurs during many neuro- and cardiovascular interventions and has recently been used to better target IA drugs to brain tumors. In the present experiments, we assessed whether the effectiveness of IA delivery of cationic liposomes could be improved by TCH.

Methods—Cationic liposomes composed of 1:1 DOTAP:PC (dioleoyl-trimethylammonium-propane:phosphatidylcholine) were administered to three groups of Sprague Dawley rats. In the first group, we tested the effect of blood flow reduction on IA delivery of cationic liposomes. In the second group, we compared TCH-assisted IA liposomal delivery vs. intravenous (IV) administration of the same dose. In the third group, we assessed retention of cationic liposomes in brain four hours after TCH assisted delivery. The liposomes contained a near infrared dye, DiIC₁₈(7), whose concentration could be measured *in vivo* by diffuse reflectance spectroscopy.

Results—IA injections of cationic liposomes during TCH increased their delivery approximately four-fold compared to injections during normal blood flow. Optical pharmacokinetic measurements revealed that relative to IV injections, IA injection of cationic liposomes during

Conflict of Interest

The authors declare that they have no conflict of interest.

TCH produced tissue concentrations that were 100-fold greater. The cationic liposomes were retained in the brain tissue four hours after a single IA injection. There was no gross impairment of neurological functions in surviving animals.

Conclusions—Transient reduction in CBF significantly increased IA delivery of cationic liposomes in the brain. High concentrations of liposomes could be delivered to brain tissue after IA injections with concurrent TCH while none could be detected after IV injection. IA-TCH injections were well tolerated and cationic liposomes were retained for at least 4 hours after IA administration. These results should encourage development of cationic liposomal formulations of chemotherapeutic drugs and their IA delivery during TCH.

Keywords

intraarterial; transient cerebral hypoperfusion; liposomes; diffuse reflectance spectroscopy; fluorescence imaging

Introduction

A recent report describes temporary occlusion of the basilar to reduce blood flow so as to better control intraarterial (IA) bevacizumab delivery while treating brain tumors [1]. Similar techniques that reduce the blood flow have been used for IA treatment of liver and breast cancers [2–4]. However, advantages of reducing blood flow during IA injections go beyond the better control of drug delivery. Pharmacokinetic models suggest that IA drug delivery would be more effective when blood flow is reduced compared to normal blood flows [5, 6]. Experimental data show that transient reduction of cerebral blood flow (CBF) can increase non-liposomal drug delivery by 5–10 fold [7–9]. Pharmacokinetic models also show that IA delivery requires rapid extraction of drug during its first pass through the cerebral circulation. For maximum benefits of IA treatments, therefore, the reduction of blood flow has to be in conjunction with rapid uptake of the drug by the brain tissue.

In recent years liposomal formulations of drugs have emerged as vehicles to improve regional drug delivery but few study have investigated their IA applications. Intraarterial (IA) delivery of cationic liposomes is attractive because the positive surface charge of the liposomes will promote their retention by binding to the negatively charged vascular endothelial cells. For example, compared to intravenous (IV) delivery, IA administration of cationic magnetic nanoparticles results in 15-fold higher tissue drug concentrations [10]. Due to the non-specific uptake of cationic liposomes by the liver and spleen, and their binding to serum proteins that attenuates their surface charge, local delivery of cationic liposomes to the brain is limited after intravenous injections. The question arises whether transiently reducing blood flow could further improve the IA delivery of cationic liposomes.

The ultra-fast first-pass kinetics of IA drug injections are beyond the temporal resolution of conventional pharmacokinetic methods, such as micro-dialysis and tissue biopsy [11]. Therefore, we used a specialized version of diffuse reflectance spectroscopy (DRS) to track brain tissue concentrations of cationic liposomes that were labeled with a near infrared dye, DiI_{C18}(7) [12–14]. In this report we first determined the effect of flow reduction on the regional deposition of cationic liposomes. Having observed a clear increase in deposition,

we compared the net benefit of such a method of drug delivery to traditional IV injections. Finally, to determine the safety of TCH assisted drug delivery and whether the increase in tissue concentrations of liposomes was sustained over time, we allowed the animals to recover from TCH assisted drug delivery and sacrificed them four hours afterwards to assess tissue liposome concentrations.

Materials and methods

After approval by the institutional animal care and use committee, the three sets of experiments were conducted on Sprague Dawley male rats weighing 200–300 g. Under anesthesia with intramuscular ketamine, supplemented with IV propofol and/or inhaled isoflurane, surgical preparation included the following: placement of a 24G tail vein cannula; tracheostomy (group 1 and 2) or an endotracheal intubation (group 3) for mechanical ventilation using a small animal ventilator (Harvard Apparatus, Holliston, MA); right femoral arterial cannulation (P-10 PTFE) and right common carotid cannulation (also P-10 PTFE); and the internal carotid artery (ICA) isolation by ligating external carotid branches. Arterial lines were flushed with de-aired heparin saline (1 unit/ml). Through a mid-line incision, the skull was exposed. The right parietal bone was thinned such that the cortical arteries and veins could be seen distinctly through the inner table and was used for monitoring brain tissue liposome concentrations. The right frontal bone was also thinned for placement of a laser Doppler probe. EKG and end-tidal gas composition were monitored continuously. A pulse oximeter was placed over the right forepaw. Esophageal and rectal probes monitored the animal's core temperature.

ICA isolation

Because of anatomical variations of the carotid system, a careful isolation of the ICA is necessary for obtaining consistent results from IA drug delivery experiments. The dissection was done by an experienced operator (MW) who first identified the vessel by its anatomy, and subsequently by the retinal discoloration test, which gives blanching of the retina upon injection of a 0.1 ml of saline bolus [15].

Diffuse reflectance spectroscopy (DRS)

Changes in back-scattered light from tissue after an injection of a chromophore can be assessed by DRS. Bigio et al. have described the underlying physics of the method in earlier publications [13, 14, 16, 17]. DRS measurements were made with a custom-built system (Optimum Technology Inc., Boston MA). A fiber optic probe, with specified separation between the emitting and collecting fibers, enabled extraction of absolute chromophore concentrations by the optical pharmacokinetic method [13, 14, 18]. A depth of ~2–4 mm of brain tissue can be interrogated with this optical geometry, and a measurement takes less than 0.5 sec. These measurements can be undertaken through the inner table of the thinned skull. A total of 612 spectra over a period of approximately 30 minutes were obtained from the brain tissue for each injection in order to generate a concentration-time curve.

Multispectral Imaging (MSI)

Surface fluorescence images of postmortem tissues were acquired by a custom built spectral imaging system similar to that described in Bouchard et. al. [19]. Excitation of the extracted whole brain utilized a 735 nm LED equipped with a 740 nm band-pass filter (Thorlabs, Newton, NJ). A 750 nm long-pass dichroic mirror was used to couple the optical paths of the light source and imager and allow fairly even illumination over field of view (Edmund Optics, Barrington, NJ). Fluorescence images were acquired by a CCD camera with a 780 ± 10 nm band-pass filter was mounted onto the lens. Image acquisition was automated by a microcontroller programmed with a custom C program that synchronized camera integration time with the LED strobe illumination settings. Matlab (MathWorks, Natick, MA) was used for analysis of the fluorescence images.

Postmortem measurements of liposome concentration in brain tissue

In survival studies investigating longer-term liposome deposition, *in vivo* DRS measurements were not possible to minimize trauma to the animals. We therefore used MSI in conjunction with DRS to determine tissue concentrations postmortem at the desired sampling time. The baseline was usually obtained from the contralateral middle- or posterior cerebral arterial distribution, which has minimal liposome exposure during IA injection. The acquired DRS spectra were normalized by the intensity at 800 nm. The concentrations of liposomes were determined by DRS in the ipsilateral and contralateral hemispheres, and in the regions of distribution of the anterior-, middle-, and posterior cerebral arteries (Fig. 3).

Micro-pulse injection

For IA injection, a total volume of 1 ml of liposomes was injected as micro-boluses of approximately 67 μ l administered at 3-second intervals using an electronically controlled pneumatic syringe system. A signal generator (33220A, Agilent Technologies Inc. Santa Clara, CA) was used to trigger the solenoid on a pressure ejector (Picospritzer III, Parker Hannifin, Pine Brook, NJ), permitting reproducible pulses of compressed air (20 PSI) to drive the plunger of the attached syringe.

Transient Cerebral Hypoperfusion (TCH)

To maximize IA drug delivery, CBF was decreased transiently during liposome injection. TCH was achieved by IV bolus injection via the tail vein of adenosine (2 mg) and esmolol (2 mg) followed by a saline flush and concurrent contralateral arterial occlusion, as needed. In rats, this produces TCH the duration of TCH can be greatly increased if the animal is rendered hypothermic (30–32 C). In the IA injection regimen, the contralateral arterial occlusion was released after drug injection, and in the hypothermic group, warming was started soon after drug injection. However, hypothermia alters mean arterial pressures, reduces CBF, and delays recovery from anesthesia. Therefore, in the second and third groups of animals, TCH was induced by flushing 2.5 ml of cold saline (4 C) via the tail vein catheter following the administration of the hypotensive drugs. In normothermic animals (group 2 and 3), TCH lasts 2–5 minutes and reverses spontaneously (Fig. 1).

Liposome preparation

The source of reagents were: dimyristoylphosphatidylcholine (DMPC) and dioleoyl-trimethylammonium-propane (DOTAP): Avanti Polar Lipids (Alabaster, AL); DiI_{C18}(7) dialkyl carbocyanine membrane label (DiR): Invitrogen (Carlsbad, CA); cholesterol (Chol): Sigma-Aldrich (St. Louis, MO).

The liposomes were prepared by hydration of a dried lipid film followed by extrusion to produce a uniform size distribution. Briefly, DMPC:DOTAP:Chol:DiI_{C18}(7) in molar ratios of 2.75:2.75:4.5:1.1 were mixed in chloroform and dried under vacuum. The resulting lipid film was hydrated to a concentration of 20 mM in NaCl/Tris (150mM NaCl, 25mM TRIS, pH 7.0) with vigorous vortexing. After 5 freeze-thaw cycles, the preparation was extruded stepwise through polycarbonate filters of decreasing pore size to a final diameter of 80 nm. The final phospholipid concentration was quantified by phosphorous assay [20], and the preparation was diluted in NaCl/Tris to a concentration of 10 μ moles of diacyl lipid (DOTAP+DMPC) per ml. Prior to injection, 0.6 ml of the liposomes were diluted in NaCl/Tris buffer to a volume of 1 ml.

Data acquisition and statistical analysis

The hemodynamic data were recorded in real-time (200 Hz) on a Power Lab data acquisition system (AD Instruments Inc., Colorado Springs, CO). Data were analyzed at: (a) baseline before injection of liposomes, (b) at the peak concentration after injection, (c) 5 min after injection, and (d) 30 min after injection. The total exposure to cationic liposomes was determined as the sum of the area under the concentration-time curve (AUC). For group 1 animals, the AUC was further divided into injection and clearance phases. Data were analyzed by ANOVA for repeated measures, and ANOVA factorial as post-hoc correction was used for multiple comparisons. Statistical Analysis was done using the Stat View 5.2 program (SAS Institute, Cary, NC).

Results

Three experiments were conducted on a total of 30 male Sprague Dawley rats. These experiments were aimed to: (i) investigate the effect of TCH during IA injection upon the tissue deposition of cationic liposomes; (ii) compare TCH-assisted IA drug delivery vs. conventional IV delivery of cationic liposomes; and (iii) investigate whether liposome deposition in brain following TCH-assisted IA delivery persists over longer time periods.

Effect of TCH on the IA delivery of cationic liposomes

Eleven animals were randomized into two treatment arms: 5 with normal CBF and 6 with TCH. In this initial experiment, the core temperature was different between the two groups because systemic hypothermia was used to achieve a reproducible TCH by inducing transient cardiac arrest (30.1 ± 1.6 °C vs. 36.5 ± 2.1 °C). As expected, physiological differences were evident between the two groups due to the background hypothermia (Table 1). Injection of liposomes during TCH increased tissue deposition. Peak liposome concentrations were significantly higher when administered with TCH compared to normal blood flow, 1.32 ± 0.51 vs. 0.72 ± 0.28 μ M, respectively, $p < 0.05$. End concentrations were

$0.43 \pm 0.17 \mu\text{M}$ for TCH vs. $0.11 \pm 0.06 \mu\text{M}$ for normal blood flow, $p < 0.005$, and total area under the concentration-time curve was considerably higher for the TCH group, Table 1, Fig. 2.

Direct comparison between TCH-assisted IA drug delivery and conventional IV delivery

In this experiment, a modified method was used to decrease the CBF: adenosine and esmolol were administered and then flushed with cold saline to induce mild hypothermia. The IV injection of cationic liposomes had no significant hemodynamic effect the hemodynamic and blood flow parameters remained stable during IV injections. With TCH assisted IA delivery, CBF decreased and subsequently returned rapidly to baseline after injection.

The observed brain concentration of liposomes after IA injection registered a clear peak that coincided with the end of the injection, whereas there was no distinct peak with IV injections. Liposome concentrations in the brain were barely detected after IV injection. The peak value of liposome deposition with flow-arrest IA delivery was 30-fold higher than observed following IV injection, and the end-value concentration of liposomes was 100 fold greater in the flow-arrest IA delivery group (Table 2, Fig. 2). However, low concentration of liposomes after IV injection generated subtle spectral changes more susceptible to noise, which may have affected DRS curve fitting results.

Longer-term retention of cationic liposomes

The objective of this group was to assess the safety of flow arrest drug delivery and to determine whether cationic liposomes were retained for extended time periods. Seven of nine animals completed the survival protocol; two animals were lost due to bleeding from the carotid stump after catheter removal: one was sacrificed at 1 hour and the other at 2 hours after liposome injection. The data from these animals was excluded from analysis and is presented separately. At four hours, the remaining seven animals had recovered from anesthesia, and were spontaneously active in their cages, with no evidence of any gross neurological impairment. Although fewer hemodynamic parameters were monitored during IA administration so as to minimize trauma, the hemodynamic changes in this group were similar to those observed in non-survival experiments: a significant decrease in heart rate, end-tidal CO_2 , and skin blood flow during injection (Table 3), all of which recovered rapidly after the administration protocol. With postmortem multispectral imaging of the extracted brain, retention of liposomes was clearly evident in the hemisphere of drug infusion four hours after administration (Fig. 3). Quantification of regional concentrations of liposomes by diffuse reflectance spectroscopy, guided by multispectral imaging, is shown in Fig. 3 and 4.

Discussion

Reduction of cerebral blood flow to assist IA drug delivery is different for brain as compared to other organs [2, 3]. The benefits of such interventions have to be balanced against the potential risk of stroke. The high resting blood flow of the brain tissue can diminish the efficacy of IA injections. It decreases peak concentrations, reduces tissue drug transit time, and increases drug washout. A reduction in bolus volume below 20% of the background blood flow can lead to regional variations in downstream drug concentrations

due to streaming [21–23]. Better targeting of drugs has led to the clinical use of arterial occlusion for treating brain tumors in human subjects [1]. While reducing blood flow can enhance the efficacy of IA drugs, their full impact will only be evident if there was rapid uptake of the drug during its first pass through the cerebral circulation [3, 5]. Therefore, there is a need to for optimal selection and formulation of IA drugs prior to clinical use.

Here we investigated whether transient reduction of CBF during IA injection would increase regional deposition of cationic liposomes, which show high extraction efficiencies in brain compared to uncharged or anionic liposomes [24]. The results suggest that uptake is increased approximately three-fold when IA injections are made during TCH compared to injections made during normal blood flow. Optical pharmacokinetic measurements revealed that the peak concentrations achieved by IA injection during cerebral hypoperfusion were 30 fold higher than those achieved by injection of the same dose IV. In addition, the end concentrations of cationic liposomes retained in the brain 30 min after injection were 100-fold higher for IA delivery compared to IV delivery. Furthermore cationic liposomes were retained by the brain tissue 4 hours after a single injection.

The concept of regional delivery of drugs by the IA route has been pursued with varying degrees of vigor over the last half century [1, 5, 25]. Although an attractive idea, IA drug delivery has not found widespread clinical application for brain tumor treatments [2, 3, 11]. A fundamental challenge to IA drug delivery is that drugs must be extracted efficiently as they transit rapidly through a very well perfused tissue. The high cerebral blood flow dilutes the injected drugs, decreases the transit time, thereby limiting contact with the endothelial cell surfaces, and washes out much of the drug that distributed into the tissue when peak concentrations were achieved. For effective uptake, drugs must be rapidly absorbed and tightly retained by the brain tissue during the short transit time through the cerebral circulation.

Drugs and drug formulations are seldom developed or screened for the IA delivery route, and thus seldom have the physicochemical characteristics necessary for efficient delivery. The results of this study that show improved regional drug deposition of cationic liposomes with IA injections, that is further enhanced delivery during TCH, are consistent with observations in other investigations. IA injection of BCNU achieves 50 fold higher concentrations in human subjects than achieved by systemic IV injections, based on positron emission tomography [26]. In rabbits subjected to IA injection of BCNU during TCH, we observed a 24-fold increase in brain tissue concentrations after 5 minutes, compared to IV injection [7]. Indirect measurement of tissue concentrations, such as by the duration of electro-cerebral silence after intracarotid injection of anesthetic drugs, similarly suggest a five- to ten-fold increase in dose effects when injected IA during TCH. The relevant observation here is that not only was there an enormous increase in tissue liposomal deposition after TCH assisted IA delivery, significant retention of cationic liposomes in the brain was observed for up to 4 hours after injection.

Manipulation of blood flow is seldom utilized as a means enhance drug delivery. However, in conjunction with IA delivery, bolus injection of drugs during TCH is both feasible and beneficial for several reasons: IA injection achieves higher peak arterial blood

concentrations, and minimizes protein binding and uptake by blood elements that reduce deposition in the target tissue; TCH increases transit time to facilitate tissue uptake, and decreases blood clearance. Under normal circumstances, brain tissue can tolerate several minutes of cerebral ischemia, which can be extended further with hypothermia. Transient blood flow reduction is often used in neurovascular surgery such as in clipping of aneurysms, endovascular procedures such as during balloon test occlusion, embolization of high flow cerebral arteriovenous malformations, and deployment of stents. At our center adenosine was used to transiently arrest cerebral blood flow in human subjects for treating high flow cerebral arteriovenous malformations [27–29]. However, small- balloon tipped microcatheters can circumvent the need for systemic arrest. Such microcatheters can reduce blood flow locally while permitting drug injections in a small region of interest as shown recently by Riina et al for human glioma treatments [1]. In 2004, Yamane et al used balloon occlusion of the supra-ophthalmic internal carotid artery in 187 pediatric patients during 563 procedures for super-selective IA delivery of chemotherapeutic drugs for retinoblastoma treatment. The only reported complication was bradycardia during injection [4]. Thus, a reasonable case can be made for employing TCH to augment drug delivery in the treatment of fatal brain diseases such as malignant brain tumors.

We have not yet evaluated the flow arrest IA delivery of the liposomes in experimental tumor models but we can foresee additional considerations in delivering them to brain tumors [30]. For example, tumor blood vessels have greater permeability that will increase liposomal extravasation. However, the tumor stroma might restrict diffusion, particularly of larger liposomes. To optimize IV liposomal delivery to tumors, liposomes are protected with poly-ethylene glycol that decreases their uptake by reticulo-endothelial cells so as to increase their circulating half lives. As a result of their extended life, the particles are preferentially taken up by tumors due to a more permeable tumor-blood barrier compared to normal tissues [31]. However, with IA delivery such protection is not needed as the particles will avoid contact with blood cells and interact directly with the endothelium. The retention of liposomes over 4 hours in the present study suggests that cationic liposomes are likely to be retained by tumors as well. However, as with any other nanoparticle, size of liposome will critical to diffusion across the blood tumor barrier and within the tumor stroma.

Conclusion

In summary, this study highlights two things. First, it highlights the approach to optimizing liposome delivery by using optical tracers as drug surrogates to permit high speed measurements by diffuse reflectance spectroscopy, supported by postmortem multispectral imaging. Second, it highlights the value of TCH assisted IA pulse injections of cationic liposomes for brain drug delivery. TCH was well tolerated by the animals and cationic liposomes were retained for at least 4 hours after IA administration. These results should encourage development of chemotherapeutic drug containing cationic liposomes and their IA delivery during TCH.

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References

1. Riina HA, Burkhardt JK, Santillan A, Bassani L, Patsalides A, Boockvar JA. Short-term clinico-radiographic response to super-selective intra-arterial cerebral infusion of Bevacizumab for the treatment of vestibular schwannomas in Neurofibromatosis type 2. *Interv Neuroradiol*. 2012; 18:127–132. [PubMed: 22681725]
2. Aigner KR. Intra-arterial infusion: overview and novel approaches. *Seminars in surgical oncology*. 1998; 14:248–253. [PubMed: 9548608]
3. Wallace S, Carrasco CH, Charnsangavej C, Richli WR, Wright K, Gianturco C. Hepatic artery infusion and chemoembolization in the management of liver metastases. *Cardiovascular and interventional radiology*. 1990; 13:153–160. [PubMed: 2121343]
4. Yamane T, Kaneko A, Mohri M. The technique of ophthalmic arterial infusion therapy for patients with intraocular retinoblastoma. *International journal of clinical oncology*. 2004; 9:69–73.10.1007/s10147-004-0392-6 [PubMed: 15108036]
5. Dedrick RL. Arterial drug infusion: pharmacokinetic problems and pitfalls. *J Natl Cancer Inst*. 1988; 80:84–89. [PubMed: 3278123]
6. Dedrick RL. Interspecies scaling of regional drug delivery. *Journal of Pharmaceutical Sciences*. 1986; 75:1047–1052. [PubMed: 3820098]
7. Joshi S, Wang M, Etu JJ, Suckow RF, Cooper TB, Feinmark SJ, Bruce JN, Fine RL. Transient cerebral hypoperfusion enhances intraarterial carmustine deposition into brain tissue. *J Neurooncol*. 2007
8. Joshi S, Wang M, Etu JJ, Pile-Spellman J. Reducing cerebral blood flow increases the duration of electroencephalographic silence by intracarotid thiopental. *Anesthesia & Analgesia*. 2005; 101:851–858. [PubMed: 16116003]
9. Joshi S, Wang M, Etu JJ, Nishanian EV, Pile-Spellman J. Cerebral blood flow affects dose requirements of intracarotid propofol for electrocerebral silence. *Anesthesiology*. 2006; 104:290–298. [PubMed: 16436848]
10. Zhao M, Chang J, Fu X, Liang C, Liang S, Yan R, Li A. Nano-sized cationic polymeric magnetic liposomes significantly improves drug delivery to the brain in rats. *J Drug Target*. 2012; 20:416–421. [PubMed: 22519867]
11. Joshi S, Meyers PM, Ornstein E. Intracarotid delivery of drugs: the potential and the pitfalls. *Anesthesiology*. 2008; 109:543–564. [PubMed: 18719453]
12. Bigio IJ, Mourant JR, Los G. Noninvasive, in-situ measurement of drug concentrations in tissue using optical spectroscopy. *J Gravit Physiol*. 1999; 6:P173–175. [PubMed: 11543008]
13. Reif R, Wang M, Joshi S, A' Amar O, Bigio IJ. Optical method for real-time monitoring of drug concentrations facilitates the development of novel methods for drug delivery to brain tissue. *Journal of biomedical optics*. 2007; 12:034036.10.1117/1.2744025 [PubMed: 17614744]
14. Mourant JR, Johnson TM, Los G, Bigio IJ. Non-invasive measurement of chemotherapy drug concentrations in tissue: preliminary demonstrations of in vivo measurements. *Physics in Medicine & Biology*. 1999; 44:1397–1417. [PubMed: 10368027]
15. Joshi S, Wang M, Hartl R. Retinal Discoloration Test. *Journal of Cerebral Blood Flow and Metabolism*. 2004; 24:305–308. [PubMed: 15091111]
16. Bigio IJ, Bown SG. Spectroscopic sensing of cancer and cancer therapy: current status of translational research. *Cancer Biol Ther*. 2004; 3:259–267. [PubMed: 15107613]
17. Mourant JR, Bigio IJ, Jack DA, Johnson TM, Miller HD. Measuring absorption coefficients in small volumes of highly scattering media: Source-detector separations for which path lengths do not depend on scattering properties. *Applied Optics*. 1997; 36:5655–5661. [PubMed: 18259392]
18. Bigio IJ, Mourant JR, Los G. Noninvasive, in-situ measurement of drug concentrations in tissue using optical spectroscopy. *Journal of Gravitational Physiology: a Journal of the International Society for Gravitational Physiology*. 1999; 6:P173–175. [PubMed: 11543008]
19. Bouchard MB, Chen BR, Burgess SA, Hillman EM. Ultra-fast multispectral optical imaging of cortical oxygenation, blood flow, and intracellular calcium dynamics. *Optics express*. 2009; 17:15670–15678.10.1364/OE.17.015670 [PubMed: 19724566]

20. Bartlett GR. Phosphorus assay in column chromatography. *J Biol Chem.* 1959; 234:466–468. [PubMed: 13641241]
21. Lutz RJ, Dedrick RL, Boretos JW, Oldfield EH, Blacklock JB, Doppman JL. Mixing studies during intracarotid artery infusions in an in vitro model. *J Neurosurg.* 1986; 64:277–283. [PubMed: 3944637]
22. Saris SC, Blasberg RG, Carson RE, deVroom HL, Lutz R, Dedrick RL, Pettigrew K, Chang R, Doppman J, Wright DC. Intravascular streaming during carotid artery infusions. Demonstration in humans and reduction using diastole-phased pulsatile administration. *Journal of Neurosurgery.* 1991; 74:763–772. [PubMed: 1849555]
23. Saris SC, Shook DR, Blasberg RG, Dedrick RL, Doppman JL, Bankiewicz KS, Blacklock JB, Oldfield EH. Carotid artery mixing with diastole-phased pulsed drug infusion. *Journal of Neurosurgery.* 1987; 67:721–725. [PubMed: 3668642]
24. Joshi S, Straubinger R, Singh-Moon R, Wang M, Rai R, Bigio I. Surface Charge Manipulation of Liposomes to Enhance Intraarterial Delivery. *J Neurosurg Anesthesiol.* 2012; 24:492–493.
25. Dedrick RL, Oldfield EH, Collins JM. Arterial drug infusion with extracorporeal removal. I. Theoretic basis with particular reference to the brain. *Cancer treatment reports.* 1984; 68:373–380. [PubMed: 6697327]
26. Mitsuki S, Diksic M, Conway T, Yamamoto YL, Villemure JG, Feindel W. Pharmacokinetics of ¹¹C-labelled BCNU and SarCNU in gliomas studied by PET. *J Neurooncol.* 1991; 10:47–55. [PubMed: 1902507]
27. Pile-Spellman J, Young WL, Joshi S, Duong DH, Vang MC, Hartmann A, Kahn RA, Rubin DA, Prestigiacomo CJ, Ostapkovich ND. Adenosine-induced cardiac pause for endovascular embolization of cerebral arteriovenous malformations: Technical case report. *Neurosurgery.* 1999; 44:881–886. discussion 886–887. [PubMed: 10201317]
28. Hashimoto T, Young WL, Aagaard BD, Joshi S, Ostapkovich ND, Pile-Spellman J. Adenosine-induced ventricular asystole to induce transient profound systemic hypotension in patients undergoing endovascular therapy: dose-response characteristics [In Process Citation]. *Anesthesiology.* 2000; 93:998–1001. [PubMed: 11020753]
29. Hashimoto T, Young WL, Davis CC, Joshi S, Ostapkovich ND. Dose-response characteristics of adenosine when used for cardiac pause for deliberate systemic hypotension (Abstract #807). *Journal of Neurosurgical Anesthesiology.* 1999; 11:327.
30. Drummond DC, Meyer O, Hong K, Kirpotin DB, Papahadjopoulos D. Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. *Pharmacol Rev.* 1999; 51:691–743. [PubMed: 10581328]
31. Drummond DC, Noble CO, Hayes ME, Park JW, Kirpotin DB. Pharmacokinetics and in vivo drug release rates in liposomal nanocarrier development. *J Pharm Sci.* 2008; 97:4696–4740.10.1002/jps.21358 [PubMed: 18351638]

Fig. 1.

Representative physiological data during transient cerebral hypoperfusion (TCH). TCH involves unilateral or bilateral occlusion of carotid arteries combined with systemic hypotension induced by exceedingly short-acting hypotensive drugs such as adenosine and esmolol. TCH was assisted by systemic hypothermia (Group 1) or cold saline injection (Groups 2 and 3). The systemic hemodynamic parameters returned to baseline within a few minutes after initiating the TCH sequence and drug injection. No inotropic drugs were required to reverse the hypotensive challenge. Solid bar indicates 5 minutes

Fig. 2.

Representative concentration-time curves and multispectral images of the whole brain showing the distribution of liposomes. Left, above: Group 1: representative time courses of cationic liposomes in brain after intracarotid (IC) delivery in the absence or presence of transient cerebral hypoperfusion (IC-TCH). Left below: Multispectral image shows liposome deposition in postmortem brain, approximately 30 min after intraarterial administration (B) Group 2. Right: Clearance curve comparing liposome deposition in brain following IC-TCH vs. normal blood flow (Group 1, top) and IC-TCH vs. intravenous (IV) delivery, bottom

Fig. 3. Multispectral image and brain tissue concentration of liposomes 4 hours after IA-TCH delivery. Figure on the left shows tissue concentrations of liposomes in different arterial irrigations 4 hours after liposome injection in the ipsilateral and contralateral cerebral hemispheres (P values are indicated by brackets at the top of the figure) and where also compared to the primary target site of drug delivery, the ipsilateral MCA. Abbreviations: ACA: anterior cerebral artery; MCA: middle cerebral artery; PCA: posterior cerebral artery. Figure on the right is a multispectral image showing the distribution of liposomes in brain tissue in one representative animal

Fig. 4.

Long-term retention of cationic liposomes following IA administration with TCH. Figure shows liposome concentrations in postmortem brain tissue from animals of Group 3. The data includes seven animals that completed the 4 hour survival protocol. Two animals were sacrificed prematurely (at 1 and 2 hours after liposome injection) because of bleeding after catheter removal. The data for these lone animals are included in the figure for comparison. ACA: anterior cerebral artery, MCA: middle cerebral artery and PCA: posterior cerebral artery

Table 1

Physiological and pharmacokinetic parameters during regional IA delivery of liposomes in the absence and presence of TCH

Group 1: IA infusion vs. IA-TCH		IA alone (n=5)	IA-TCH (n=6)
<i>Peak concentration (μM)</i>		0.72 $\pm$ 0.28*	1.32 $\pm$ 0.51
<i>AUC injection ($\mu\text{M}\cdot\text{s}$)</i>		27.1 $\pm$ 12.1*	73.3 $\pm$ 28.3
<i>AUC clearance ($\mu\text{M}\cdot\text{s}$)</i>		270.3 $\pm$ 113.5*	421.2 $\pm$ 249.1
<i>AUC total ($\mu\text{M}\cdot\text{s}$)</i>		297.3 $\pm$ 119.7*	1169.6 $\pm$ 502.0
<i>End Concentration (μM)</i>		0.11 $\pm$ 0.06*	0.43 $\pm$ 0.17

	Challenge	Baseline	Peak injection	5min	30min
Temperature (C)	IA	36.5 $\pm$ 2.1	36.2 $\pm$ 1.9	36.6 $\pm$ 1.6	37.5 $\pm$ 1.7
	IA-TCH	30.1 $\pm$ 1.6	29.7 $\pm$ 1.3	29.9 $\pm$ 1.4	30.8 $\pm$ 1.5
Heart rate, (bpm)	IA	241 $\pm$ 44	249 $\pm$ 39	251 $\pm$ 38	245 $\pm$ 42
	IA-TCH	180 $\pm$ 19	98 $\pm$ 30	190 $\pm$ 25	221 $\pm$ 39
ETCO2 (mmHg)	IA	34 $\pm$ 7	34 $\pm$ 7	34 $\pm$ 6	34 $\pm$ 6
	IA-TCH	23 $\pm$ 3	9 $\pm$ 3	23 $\pm$ 5	26 $\pm$ 4
MAP (mmHg)	IA	63 $\pm$ 7	64 $\pm$ 5*	61 $\pm$ 3*	61 $\pm$ 7
	IA-TCH	52 $\pm$ 6	21 $\pm$ 7	45 $\pm$ 14	49 $\pm$ 12
% CBF	IA	100 $\pm$ 0	89 $\pm$ 13*	98 $\pm$ 7*	100 $\pm$ 8
	IA-TCH	100 $\pm$ 0	26 $\pm$ 22	49 $\pm$ 34	80 $\pm$ 41

* significant post-hoc difference from IA-TCH, factorial ANOVA. IA, intraarterial; TCH, transient cerebral hypoperfusion.; AUC, area under curve; ETCO2, end-tidal carbon dioxide tension; MAP, mean arterial pressure; CBF, cerebral blood flow. There were significant physiological differences between IA (normothermic) and IA-TCH (hypothermic groups)

Table 2

Physiological and pharmacokinetic parameters during IA administration with TCH vs. systemic IV administration of liposomes

Group 2: IV Bolus vs. IA-TCH		IV (n=5)	IA-TCH (n=5)		
Peak concentration (μM)		0.02±0.01 *	1.59±0.41		
AUC total (μM·s)		9.1±7.1 *	927.9±350.9		
End Concentration (μM)		0.00±0.01 *	0.43±0.22		
	Challenge	Baseline	Peak injection	5min	30min
Heart rate, (bpm)	IV	269±39	274±35 *	303±22	314±20
	IA-TCH	295±47	67±64	285±15	301±45
ETCO2 (mmHg)	IV	32±5	32±5	31±4	34±5
	IA-TCH	32±5	6±2	32±7	32±4
MAP (mmHg)	IV	55±6	57±17 *	59±16	58±9
	IA-TCH	68±17	18±3	69±8	70±13
% CBF	IV	100±0	100±9 *	109±11	132±40
	IA-TCH	100±0	5±4	104±34	95±15

* significant post-hoc difference from IA-TCH, factorial ANOVA. IA, intraarterial; TCH, transient cerebral hypoperfusion; IV, intravenous; AUC, area under curve; ETCO2, end-tidal carbon dioxide tension; MAP, mean arterial pressure; CBF, cerebral blood flow

Physiological parameters during regional IA delivery of liposomes and deposition in ipsilateral vs. contralateral hemispheres in 4 hr survival experiment

Table 3

Group 3: Tissue concentrations		Ipsilateral	Contralateral
N=7		cerebral hemisphere	cerebral hemisphere
Concentration ACA (μ M)		0.13 $\pm$ 0.17 (P=0.04)	0.06 $\pm$ 0.06
Concentration MCA (μ M)		0.17 $\pm$ 0.06 (P=0.004)	0.07 $\pm$ 0.07
Concentration PCA (μ M)		0.16 $\pm$ 0.06 (P=0.009)	0.07 $\pm$ 0.04

	Challenge	Baseline	Peak	5min	30min
Heart rate (bpm)	IA-TCH	270 $\pm$ 44 [*]	58 $\pm$ 23	287 $\pm$ 30 [*]	310 $\pm$ 11 [*]
ETCO2 (mmHg)	IA-TCH	37 $\pm$ 8 [*]	8 $\pm$ 2	36 $\pm$ 9 [*]	40 $\pm$ 9 [*]
% Skin blood flow	IA-TCH	100 $\pm$ 0 [*]	12 $\pm$ 6	74 $\pm$ 33 [*]	114 $\pm$ 71

* significant post-hoc difference from peak, repeat measure ANOVA. IA, intraarterial; ACA, anterior cerebral artery; MCA, middle cerebral artery; PCA, posterior cerebral artery; ETCO2, end-tidal carbon dioxide tension, TCH, transient cerebral hypoperfusion