

Editorial

8-to-9 nm Size Exteriorly Anionic Dense [Gd³⁺DOTA]₂-Conjugated Nanoparticles of Dendritic Architecture with Labilely Conjugated Small Molecule Chemoxenobiotics on the Interior for Effective Passively Selective Drug Delivery into the Solid Interstitium and Concomitant Quantitative Dynamic Contrast-Enhanced MRI

Sarin H*

Freelance Investigator in Translational Science and Medicine, USA

*Corresponding author: Sarin H, Freelance Investigator in Translational Science and Medicine, 833 Carroll Road, Charleston, WV, USA

Received: August 31, 2016; Accepted: October 28, 2016; Published: November 01, 2016

Editorial

Small biomolecule therapeutics remains the cornerstone for treatment of human disease states, mostly due to their oral (gastro-intestinal) and inhaled (pulmonary) bioavailability across inter-epithelial junctional pore complexes. As such, the determinants of passively-selective inter-epithelial junctional pore complex permeation have recently been determined to be the conserved biophysical properties of the biomolecules [1], for less hydrophilic hydrophiles and lipophiles the limiting conserved biophysical property is primarily the Van Der Waals Diameter (vdWD), and for more hydrophilic hydrophiles the conserved biophysical properties are relative hydrophilicity and the vdWD: (i) Less hydrophilic hydrophiles and lipophiles are inter-epithelial junctional pore complex permeable to a vdWD of at least 0.81 nanometers (nm) [1], and upon entering systemic circulation, those > 0.78 nm in diameter interact with intravascular proteins +/- non-channel receptor proteins or directly with cell membranes, while those < 0.78 nm in diameter further permeate across cell membrane channel pores [2,3]; whereas, (ii) More hydrophilic small biomolecules are inter-epithelial junctional pore complex permeation restricted [1], as is the case of non-chelating anionic hydrophiles as well as chelating hydrophiles with maintained circumferential anionicity such as [Gd³⁺DOTA]₂-, a strong cyclic Gd³⁺ chelator and extracellular MRI contrast agent [4], with a predicted vdWD of 0.88 nm and a predicted Log *P* of -15.50 at gastric pH < 4 (pH < 4 Log *P*ow-to-vdWD ratio of -17.54 nm⁻¹) and a predicted Log *P*ow of -10.7 at intestinal pH > 4 (pH > 4 Log *P*ow-to-vdWD ratio of -12.11 nm⁻¹), which when administered intravenously is self-non-interacting as well as non-interacting with the biological system, and permeates across less permeaselective pores [1,5], for example, across the less permeaselective renal podocyte inter-epithelial junctional pore complexes (upper limit of pore size 2-to-3 nm) and across the lesser permeaselective skeletal and cardiac

muscle blood capillary macula occludens junctional pore complexes (upper limit of pore size 4-to-5 nm) as well as the least permeaselective endocrine and exocrine blood capillary diaphragm fenestrated trans-endothelial pores (upper limit of pore size 6-to-7 nm) [5], which are physiological VEGF concentration-induced diaphragm fenestrated blood capillaries [6].

As it has been long known that local inflammatory mediators such as VEGF and bradykinin, present at supra-physiologic local concentrations in the *milieu* diseased tissues, induce the co-opted neoangiogenesis of hyper-permeable blood capillaries in diseased tissues (i.e. solid tumors) [6-10], in the 1990s there had been an interest in developing clinical useful systemic intra-arterial and intravenous vasomodulation strategies employing bradykinin [11,12] and its synthetic proteolytically-resistant analogue labradimil (RMP-7, aka Cereport) [13-15] to temporarily increase the permeability-surface area product of solid tumor blood capillaries to achieve greater drug concentration of small molecule chemotherapeutics across tumor blood capillary barriers, the Central Nervous System (CNS) solid tumor Blood-Brain Tumor Barrier (BBTB) and the non-CNS solid tumor Blood-Tumor Barrier (BTB). Henceforth, vasomodulator effectiveness at increasing small molecule chemotherapeutic or surrogate small molecule accumulation in tumor tissue has been studied in rodent solid tumor models by Quantitative Autoradiography (QAR) [11-14], a methodology that requires harvesting of tumor tissue to determine the experimental endpoint tumor concentration of a bolus-infused radiolabeled small molecule chemotherapeutic (i.e. ¹⁴C-carboplatin) or a radio labeled small molecule chemotherapy surrogate [i.e. alpha-Aminoisobutyric Acid (¹⁴C-AIB)] after the systemic co-infusion of a vasomodulator at a constant rate with frequent blood plasma concentration measurements throughout the vasomodulator infusion period for integrated determination of the influx rate constant (*K_i*; *min-1*) at the experimental endpoint *vis a vis* bi-compartmental (intravascular space, intracellular space) uni-directional uni-parameter pharmacokinetic modeling. The major pitfall of QAR is accurate determination of the blood plasma pharmacokinetic curve in the setting of vasomodulator-induced hemodynamic instability (personal observation), and as such, the increase in tumor tissue radioisotope concentration (compared non-vasomodulated controls) has been attributed to an increase in vasomodulator-induced local BBTB or BTB permeability.

Model-based T_1 -weighted quantitative dynamic-contrast enhanced MRI (qDC-E MRI) has been extensively utilized for modified Toft's bi-compartmental (intravascular plasma space, extracellular space) bi-directional 4-parameter pharmacokinetic modeling of K_{trans} (forward rate constant, min^{-1}), K_{ep} (reverse rate constant, min^{-1}), V_e (tumor tissue extra-vascular extracellular space, fract) and V_p (tumor tissue vascular blood plasma volume, fract) [16,17]. In more recent years, the T_1 -weighted qDC-E MRI-modified Toft's model-derived K_{trans} for [Gd3+DTPA] $_2$ - has been validated against the QAR-derived K_i for 14C-AIB in the orthotopic rodent glioma-2 (RG-2 glioma) model [18], an anaplastic intra-cranial tumor which is histologically homogeneous in character similar to a WHO Grade III glioma [19]. Building on this finding, the Toft's model model-based [Gd3+DTPA] $_2$ - T_1 -weighted qDC-E MRI with residual contrast correction (for the first contrast agent bolus decay) has been utilized to investigate the conserved basis for vasomodulator-induced increases in BBTB permeability to [Gd3+DTPA] $_2$ - during infusions of various bradykinins such bradykinin itself and labradimil (with each glioma-bearing rodent subject serving as its own control) [20], and it has been determined that the observed vasomodulator-induced increases in BBTB permeability and tumor tissue accumulation of small molecules is due to systemic vasodilation and a decrease in blood pressure, which results in a decrease in renal filtration fraction and in a concomitant increase in the blood plasma half-life of small molecules such as [Gd3+DTPA] $_2$ - [20].

[Gd3+DTPA/DOTA] $_2$ - conjugated polyamidoamine (PAMAM) Ethyldiamine (EDA) and Diaminobutane (DAB) core dendrimer nanoparticle-based contrast agents (~1.5 nm diameter Gd-Generation 1 to 13+/-1 nm diameter Gd-Generation 8) have been utilized for non-quantitative DC-E MRI-based detection of microvascular permeability alterations in small animal models [21-23], and 5-to-6 nm size low-molecular weight (~36 kDa) PAMAM dendrimer nanoparticles have been utilized for evaluation of therapeutic potential in the same [24-27], however the optimal biophysical properties of these hydration-resistant dendritic conjugate nanoparticles including the optimal size range for effective drug delivery had not been realized until a few years later. Between mid-2006 and early-2008, the following were determined: (i) There is more focal RG-2 glioma tumor tissue distribution of the non-dense lowly conjugated 24 kDa Gd-Generation 4 dendrimer (LC Gd-G4, ~30% conjugated; 5-to-6 nm diameter) as per a lower modeled tumor tissue extra-vascular extracellular space (V_e), which does not possess a sufficiently long blood plasma half-life due to its renal clearance as it is subject to proteolytic degradation (less dense 24 kDa, 5-to-6 nm) while it has diffusion-limited tumor tissue distribution due to its 5-to-6nm size [28,29]; (ii) Model-based T_1 -weighted qDC-E MRI fails at the standard conjugated 40 kDa Gd-G4 dendrimer (Std Gd-G4, ~50% conjugated; 5-to-6 nm diameter) as the blood plasma pharmacokinetic curve approaches constancy due to decreased systemic proteolytic degradation of the Std Gd-G4 dendrimer (more dense 40 kDa, 5-to-6 nm diameter) [8,29]; (iii) (a) Non-model based T_1 -weighted qDC-E MRI without subsequent pharmacokinetic modeling can be used to accurately assess the accumulation over time of various sized [Gd3+DTPA] $_2$ -conjugated dendrimer nanoparticles, Gd-G4 to Gd-G8 (5-to-14 nm), within the tumor tissue interstitium, which simply generates voxel-by-voxel Gd concentration-maps based on the post-contrast enhancement R_1 -to-pre-contrast enhancement

R_0 ratio adjusted by $mM \cdot sec/r_1$, where R (1/sec) is the *in vivo* dual-flip angle Fast-Field Echo (FFE)-determined longitudinal relaxivity and where r_1 (1/ $mM \cdot sec$) is the *in vitro* Spin Echo (SE)-determined contrast agent molar relaxivity (*wrt* Gd), in which case conjugated chelated Gd (mM) accumulation can be measured voxel-by-voxel in RG-2 glioma solid tumor interstitium for the various sized Gd-dendrimers over prolonged duration (≥ 2 hours) [28-31]; (iii) (b) The upper limit of pore size of the BBTB and the BTB is at 12 nm, between the size of the Gd-Gd-7 dendrimer (~11+/-1 nm diameter), which is permeable across both the BBTB and the BTB, and the size of the Gd-G8 dendrimer (~13+/-1 nm diameter), which is restricted-to-permeation across both the BBTB and the BTB [28,30]; and (iii) (c) The most effective size range for tumor interstitium accumulation is the 8-to-9 nm size range (Gd-G5; ~85 kDa, est at 8.5 nm diameter; blood plasma $t^{1/2}$ 6-8 hrs) due to optimal blood plasma half-life in context of optimal permissive BBTB and BTB permeation irrespective of tumor volume [28-30], whereby the 8-to-9 nm size Gd-G5 dendrimer is passively selective for the solid tumor interstitium across the BBTB and the BTB over other normal tissues supplied by diaphragm fenestrated blood capillary [5,32].

Based on the above observations, the 8-to-9 nm size Gd-G5 dendrimer nanoparticle is the optimal size for passively selective accumulation in the solid tumor interstitium, irrespective of solid tumor host site, which therefore has been selected for the purpose of developing a theranostic dendrimer nanoparticle for quantifiable imageable effective transvascular delivery of small molecule chemotherapeutics into the solid tumor interstitium. As such, a prototype Gd-G5 dendrimer-based theranostic nanoparticle has been developed to explore therapeutic potential of such a dendritic conjugate, in which a ~50% [Gd3+DTPA] $_2$ - conjugated dendrimer has been additionally ~10% conjugated with doxorubicin (0.95 nm diameter) via labile covalent hydrazone bonds (~0.5 nm) to free exterior amides resulting in a ~9.5 nm (hydrodynamic diameter, H_D) exteriorly cationic Gd-G5 theranostic dendrimer nanoparticle [29,33]. Even as this exteriorly cationic Gd-G5 theranostic dendrimer is in the Gd-G6 dendrimer size range (which accumulates to lesser extent in tumor tissue than the Gd-G5) [28-30], when administered at the standard 0.09 mmol Gd/kg body weight (~100 mg dendrimer/ kg body weight) dose, the prototype Gd-G5 theranostic dendrimer being cationic, accumulates within orthotopic RG-2 glioma solid tumor tissue [29,33] in the accumulation range for the non-chemotherapy conjugated Gd-G5 dendrimer nanoparticles (100-300 μM *wrt* conjugated chelated Gd and 1 μM -3 μM *wrt* the Gd-G5 dendrimer nanoparticle itself) [28-30], wherein the concentration of doxorubicin in the tumor interstitium can be estimated to be within the 10 μM -30 μM range, that which results in almost complete orthotopic RG-2 glioma tumor regression after 1 dose within 24 hours [29,33], however, with significant systemic toxicity that precludes longer-term evaluation/treatment [29,33] in contrast to its exteriorly anionic non-chemotherapy conjugated Gd-G5 dendrimer counterpart, which when administered intravenously permits longer-term evaluation of rodent subjects [29,30].

In closing, it can be asserted that systemically administered nanoparticles with exterior cationicity and interior anionicity are systemically cationotoxic irrespective of size range, in contrast to those with exterior anionicity and interior cationicity that are

systemically non-toxic due to no self-self and no biological system interactions and can be accurately quantitatively assessed by qDC-E MRI [31]. As exteriorly anionic and dense nanoparticles of dendritic architecture are approved for pre-clinical use being systemically non-toxic (i.e. Gadomer-17, aka Gd-DOTA 17 dendritic conjugate; 17 kDa) [34] and resistant to proteolytic degradation, respectively, 8-to-9 nm size exteriorly anionic dense [Gd³⁺DOTA]₂- conjugated nanoparticles of dendritic architecture with labilely conjugated small molecule chemoxenobiotics on the interior have the potential to be curative due to passively selective permeation across hyper-permeable blood capillary barriers of diseased tissues with an upper limit of pore size at 12 nm (i.e. BBTB, BTB) in context of a prolonged blood plasma half-life for effective accumulation within diseased tissue interstitia [35].

References

- Sarin H. Permeation thresholds for hydrophilic small biomolecules across microvascular and epithelial barriers are predictable on the basis of conserved biophysical properties. *In Silico Pharmacology*. 2015; 3.
- Sarin H. Pressuromodulation at the cell membrane as the basis for small molecule hormone and peptide regulation of cellular and nuclear function. *Journal of translational medicine*. 2015; 13.
- Sarin H. Conserved molecular mechanisms underlying the effects of small molecule xenobiotic chemotherapeutics on cells. *Molecular and Clinical Oncology*. 2016; 4: 326-368.
- Ishiguchi T, Takahashi S. Safety of gadoterate meglumine (Gd-DOTA) as a contrast agent for magnetic resonance imaging: results of a post-marketing surveillance study in Japan. *Drugs in R&D*. 2010; 10: 133-145.
- Sarin H. Physiologic upper limits of pore size of different blood capillary types and another perspective on the dual pore theory of microvascular permeability. *Journal of angiogenesis research*. 2010; 2.
- Kamba T, Tam BYY, Hashizume H, Haskell A, Sennino B, Mancuso MR, et al. VEGF-dependent plasticity of fenestrated capillaries in the normal adult microvasculature. *Am J Physiol Heart Circ Physiol*. 2006; 290: 560-576.
- Folkman J. Tumor angiogenesis: therapeutic implications. *New England Journal of Medicine*. 1971; 285: 1182-1186.
- Jain RK. Transport of molecules across tumor vasculature. *Cancer Metastasis Rev*. 1987; 6: 559-593.
- Roberts WG, Palade GE. Neovasculature induced by vascular endothelial growth factor is fenestrated. *Cancer Research*. 1997; 57: 765-772.
- Maeda H. Vascular permeability in cancer and infection as related to macromolecular drug delivery, with emphasis on the EPR effect for tumor-selective drug targeting. *Proceedings of the Japan Academy Series B, Physical and biological sciences*. 2012; 88: 53-71.
- Inamura T, Black KL. Bradykinin selectively opens blood-tumor barrier in experimental brain tumors. *J Cereb Blood Flow Metab*. 1994; 14.
- Weyerbrock A, Walbridge S, Pluta RM, Saavedra JE, Keefer LK, Oldfield EH. Selective opening of the blood-tumor barrier by a nitric oxide donor and long-term survival in rats with C6 gliomas. *Journal of Neurosurgery*. 2003; 99: 728-737.
- Bartus RT, Snodgrass P, Dean RL, Kordower JH, Emerich DF. Evidence that Cereport's ability to increase permeability of rat gliomas is dependent upon extent of tumor growth: implications for treating newly emerging tumor colonies. *Exp Neurol*. 2000; 161: 234-244.
- Bartus RT, Snodgrass P, Marsh J, Agostino M, Perkins A, Emerich DF. Intravenous Cereport (RMP-7) modifies topographic uptake profile of carboplatin within rat glioma and brain surrounding tumor, elevates platinum levels, and enhances survival. *J Pharmacol Exp Ther*. 2000; 293: 903-911.
- Warren K, Gervais A, Aikin A, Egorin M, Balis FM. Pharmacokinetics of carboplatin administered with lobradimil to pediatric patients with brain tumors. *Cancer Chemother Pharmacol*. 2004; 54: 206-212.
- Tofts PS, Kermode AG. Measurement of the blood-brain barrier permeability and leakage space using dynamic MR imaging. *Fundamental concepts*. *Magn Reson Med*. 1991; 17: 357-3367.
- Tofts PS, Brix G, Buckley DL, Evelhoch JL, Henderson E, Knopp MV, et al. Estimating kinetic parameters from dynamic contrast-enhanced T₁-weighted MRI of a diffusible tracer: standardized quantities and symbols. *J Magn Reson Imaging*. 1999; 10: 223-232.
- Ferrier MC, Sarin H, Fung SH, Schatlo B, Pluta RM, Gupta SN, et al. Validation of dynamic contrast-enhanced magnetic resonance imaging-derived vascular permeability measurements using quantitative autoradiography in the RG2 rat brain tumor model. *Neoplasia*. 2007; 9: 546-555.
- Aas AT, Brun A, Blennow C, Stromblad S, Salford LG. The RG2 rat glioma model. *J Neurooncol*. 1995; 23: 175-183.
- Sarin H, Kanevsky AS, Fung SH, Butman JA, Cox RW, Glen D, et al. Metabolically stable bradykinin B2 receptor agonists enhance transvascular drug delivery into malignant brain tumors by increasing drug half-life. *Journal of translational medicine*. 2009; 7: 33.
- Kobayashi H, Reijnders K, English S, Yordanov AT, Milenic DE, Sowers AL, et al. Application of a macromolecular contrast agent for detection of alterations of tumor vessel permeability induced by radiation. *Clin Cancer Res*. 2004; 10: 7712-7720.
- Kobayashi H, Brechbiel M. Nano-sized MRI contrast agents with dendrimer cores. *Adv Drug Deliv Rev*. 2005; 57: 2271-2286.
- Bryant LH, Brechbiel MW, Wu C, Bulte JW, Herynek V, Frank JA. Synthesis and relaxometry of high-generation (G=5,7,9, and 10) PAMAM dendrimer-DOTA-gadolinium chelates. *J Magn Reson Imaging*. 1999; 9: 348-352.
- Jackson C, Chanzy H, Booy F, Drake B, Tomalia D, Bauer B, et al. Visualization of dendrimer molecules by transmission electron microscopy (TEM): Staining methods and cryo-TEM of vitrified solutions. *Macromolecules*. 1998; 31: 6259-6265.
- Tomalia DA, Frechet JM. Discovery of dendrimers and dendritic polymers: a brief historical perspective. *Journal of Polymer Science, Part A: Polymer Chemistry*. 2002; 40: 2719-2728.
- Tomalia DA, Reyna LA, Svenson S. Dendrimers as multi-purpose nanodevices for oncology drug delivery and diagnostic imaging. *Biochem Soc Trans*. 2007; 35: 61-67.
- Kukowska-Latallo J, Candido K, Cao Z, Nigavekar S, Majoros I, Thomas T, et al. Nanoparticle targeting of anticancer drug improves therapeutic response in an animal model of human epithelial cancer. *Cancer Res*. 2005; 65: 5317-5324.
- Sarin H, Kanevsky AS, Wu H, Brimacombe KR, Fung SH, Sousa AA, et al. Effective transvascular delivery of nanoparticles across the blood-brain tumor barrier into malignant glioma cells. *Journal of translational medicine*. 2008; 6: 80.
- Sarin H. Effective transvascular delivery of chemotherapy into cancer cells with imageable nanoparticles in the 7 to 10 nanometer size range. Slevin M, editor. *In: Current Advances in the Medical Application of Nanotechnology*: Bentham Science Publishers Ltd. 2012; 10-24.
- Sarin H, Kanevsky AS, Wu H, Sousa AA, Wilson CM, Aronova MA, et al. Physiologic upper limit of pore size in the blood-tumor barrier of malignant solid tumors. *Journal of translational medicine*. 2009; 7: 51.
- Sarin H. Translational theranostic methodology for diagnostic imaging and the concomitant treatment of malignant solid tumors. *Neurovascular Imaging*. 2015; 1.
- Sarin H. Overcoming the challenges in the effective delivery of chemotherapies to CNS solid tumors. *Therapeutic delivery*. 2010; 1: 289-305.
- Sarin H. Recent progress towards development of effective systemic chemotherapy for the treatment of malignant brain tumors. *Journal of*

- translational medicine. 2009; 7.
34. Misselwitz B, Schmitt-Willich H, Ebert W, Frenzel T, Weinmann HJ. Pharmacokinetics of Gadomer-17, a new dendritic magnetic resonance contrast agent. *Magma*. 2001; 12: 128-134.
35. Sarin H. On the future development of optimally-sized lipid-insoluble systemic therapies for CNS solid tumors and other neuropathologies. *Recent patents on CNS drug discovery*. 2010; 5: 239-252.