Journal of Controlled Release 206 (2015) 49–57

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Remote control of the permeability of the blood–brain barrier by magnetic heating of nanoparticles: A proof of concept for brain drug delivery

Seyed Nasrollah Tabatabaei [a,b], Hélène Girouard [b], Anne-Sophie Carret [c,d], Sylvain Martel [a,e,*]

[a] Institute of Biomedical Engineering, Polytechnique de Montréal, Montréal, Canada
[b] Department of Pharmacology, Faculty of Medicine, Université de Montréal, Montréal, Canada
[c] Departement of Pediatrics, Faculty of Medicine, Université de Montréal, Montréal, Canada
[d] Pediatric Hematologist-Oncologist at CHU Sainte-Justine Hospital, Montréal, Canada
[e] Department of Computer and Software Engineering, Polytechnique de Montréal, Montréal, Canada

ARTICLE INFO

Article history:
Received 29 September 2014
Received in revised form 5 February 2015
Accepted 17 February 2015
Available online 25 February 2015

Keywords:
Magnetic nanoparticles
Hyperthermia
Alternating current
Blood–brain barrier
Drug delivery

ABSTRACT

Despite advances in neurology, drug delivery to the brain remains a substantial challenge. This is mainly due to the insurmountable and selective nature of the blood–brain barrier (BBB). In this study, we show that the thermal energy generated by magnetic heating (hyperthermia) of commercially available magnetic nanoparticles (MNPs) in the brain capillaries of rats can transiently increase barrier permeability. Here, the fluorescent Evans Blue (EB) dye was used to verify the BBB integrity. Results indicate a substantial but reversible opening of the BBB where hyperthermia is applied. Also, in this investigation, analysis of CD68 immunoreactivity, an indicator of inflammation, implies that this technique is not associated with any inflammation. We have previously investigated theranostic (therapeutic and diagnostic) capabilities of the MNPs, therefore, the findings presented in this investigation are particularly encouraging for a novel targeted drug delivery system to the brain.

1. Introduction

There has been a lot of ink given to the enormous progress that scientists around the world have made in the treatment of brain related disorders, yet drug delivery to the brain remains a challenge [1]. This is mainly due to the insurmountable and selective nature of the blood–brain barrier (BBB). This barrier consists of tightly interconnected endothelial cells that circumferentially form the interior lining of the cerebral vessel walls and prevents harmful substances from entering into the brain. By extension, most drug molecules and available therapeutics are not able to reach the brain. According to Pardridge [2], more than 98% of small molecules including therapeutics do not cross this barrier. The objective of the present investigation is to provide proof that magnetic heating (hyperthermia) of magnetic nanoparticles (MNPs) by a low radiofrequency (RF) field can increase BBB permeability without perturbing other brain cells. In order to make this proposition a clinically viable technique, it is first essential to show that the opening of the BBB is local and it is entirely reversible.

Recent literature is unanimous that improving access to the BBB holds substantial potential of improving therapeutic response to various brain related disorders including brain tumors [3]. In that regard, MNPs have been vastly researched for therapeutic and diagnostic purposes [4,13,12] as well as drug delivery to the brain [5–7]. The synergy between the current study and the ongoing research on MNPs could be pivotal in bringing more effective drug delivery technique with less toxicity to the clinic and to the market.

The permeability of the BBB between adjacent endothelial cells (tight junctions) is known to increase in response to physiologically relevant temperature increase (38–39 °C) [8,9]. We have previously observed reversible disruption of the BBB in this temperature range on the surface of the brain [10]. For the first time in this study, we examined BBB permeability at the presence of moderate heat dissipated from magnetic heating of MNPs. The physics behind magnetic heating is beyond the scope of this paper, but in summary, a low radiofrequency (RF) field can magnetically excite MNPs to release an energy in the form of heat to their surroundings through a mechanism called Néel relaxation [11]. Basically, each time the magnetic moments of the MNPs align with the alternating direction of the RF field, they release this energy once they relax back to their original direction. The present investigation focuses on the influence of magnetic heating of MNPs on BBB permeability in anesthetized rats. This preliminary experiment provides evidence that our proposed approach goes beyond former methods to access the brain tissue with higher spatial precision, advanced control and lower immune reaction.

* Corresponding author at: Institute of Biomedical Engineering, Polytechnique de Montréal, Montréal, Canada.
E-mail address: sylvain.martel@polymtl.ca (S. Martel).

http://dx.doi.org/10.1016/j.jconrel.2015.02.027
0168-3659/

2. Material and methods

2.1. Characterization of the MNPs

MNPs with eight different coatings dispersed in separate aqueous solutions were kindly provided by Chemicell GmbH (Berlin, Germany). A small drop of diluted suspension from each sample was positioned on a 200-mesh carbon film and dried by air. TEM images were acquired on a Jeol JEM-2100 field-emission gun (École Polytechnique de Montréal, Canada) operated at 200 kV (see Supplementary Information Fig. S2). The crystalline structure of the MNPs was analyzed by an X-ray diffractometer (X'Pertmodel from Philipps) using a Cu-Ka radiation (λ of 1.541 Å) at 50 kV and 40 mA (see Supplementary Information Fig. S5). The superparamagnetic properties and magnetization of the MNPs were measured using a vibrating sample magnetometer (VSM) device (EV5, Magnetics, Université de Montréal, Canada) at room temperature (see Supplementary Information Fig. S3).

2.2. Magnetic heating by radiofrequency (RF) field

In this study, the RF field was set to 7.6 kA/m at 150 kHz by a 2-kW HotShot induction heating amplifier (Ameritherm Inc., New York). To apply this field, a custom-made coil was specifically designed to generate the required field strength and frequency fitted for the head of the animal as described in Supplementary Information S1. In order to determine which commercially available MNPs would provide higher heating profile, first various coatings of the MNPs were individually placed inside the RF field (see Supplementary Information Fig. S1). The temperature rise for every sample was then recorded in respect to time. Finally, the sample with the best heating profile was chosen for the in-vivo experiments.

2.3. Animal protocol

All animal procedures were performed according to the guidelines approved by the Ethics and Experimentation on Animal Committee (CDEA) of the University of Montreal. Twenty four pathogen-free Sprague–Dawley rats (250–350 g) were examined to confirm the efficacy of the proposed approach. The animals were randomly separated into five groups, namely i) Control (n = 4), ii) Control RF (n = 4), iii) Normothermia (n = 4), iv) Hyperthermia (n = 8) and v) Recovery (n = 4). The appropriate exact protocol sequence for each group is tabulated in Table 1.

- Normothermia: Anesthetized animals (2.5% isoflurane in Oxygen) in this group (n = 4) received a 0.3 mL intravenous 2% Evans Blue (EB) dye (Sigma, USA) via the tail vein. The dye was then circulated in the vasculature for 30 min. Each animal was then placed supinely on a platform at 37 °C. Then the left common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) were carefully isolated (see in Fig. 1) after a 1 cm incision in the middle of the neck. The distal portion of the ECA was permanently ligated with a 4-0 suture. Microvascular clips were applied to the CCA and the proximal parts of the ECA and ICA, and an incision was made between the ECA ligation site and the ECA clip. A 32G catheter (SAI, Illinois USA) was inserted into the ECA towards the CCA then the ICA while removing the microvascular clips. The distance between the middle cerebral artery (MCA) and the bifurcation between ECA and ICA is about 2 cm. Once the catheter was in place, an average of 60 μL poly(maleic acid-co-olefin) or PMO-coated MNPs (concentration: 12 mg Fe_3O_4/mL double distilled water) was slowly injected. After injection, the catheter was slowly retrieved and the ECA was permanently occluded to avoid excessive blood loss. The animal was then kept alive under anesthesia for 30 min. Quickly after, the animal was sacrificed via intra-cardiac perfusion of 60 cc warm 0.9% saline followed by 100 cc 4% paraformaldehyde (PFA) for the first 3 animals (RFU analysis) and 2% glutaraldehyde (stronger preservative for TEM analysis) for the last animal, to flush all the blood and EB dye out of the vascular system. Once perfusion was completed, the brain of the animal was extracted and placed inside a preservative solution (4% PFA or 2% glutaraldehyde respectively).
- Hyperthermia: An average of 60 μL PMO-coated MNPs was injected into the left MCA as described for Normothermia. Each animal was then exposed to the RF field for 30 min. The animals were then sacrificed by an intra-cardiac perfusion of 60 cc 0.9% saline followed by 100 cc 4% PFA for 7 animals (RFU analysis (n = 3) and immunohistochemistry (n = 4)) and 2% glutaraldehyde for the last animal (TEM analysis).
- Recovery: Animals in this group (n = 4) received the same PMO-coated MNP dosage via the left MCA as discussed before. They were then exposed to the RF field for 30 min and kept alive under anesthesia for 2 h. Then the animals were infused by 0.3 mL EB dye via the tail vein. After 30 min, the animals were sacrificed via an intra-cardiac perfusion as explained in Normothermia.
- Control and Control RF: Animals without MNP injection in the Control RF group (n = 4) were exposed to the RF field for 30 min and sacrificed by intra-cardiac perfusion as described in Normothermia. Animals in the Control group (n = 4) did not receive MNPs and were not exposed to the RF field. These animals were sacrificed via an inter-cardiac perfusion 30 min after the dye was introduced in the vasculature.

Table 1
Groups of animals and the appropriate procedure.

i) Control	:	EB	CP			
ii) RF control	:	EB	H_2O-dd	RF field	CP	
iii) Normothermia	:	EB	MNPs	CP		
iv) Hyperthermia	:	EB	MNPs	RF field	CP	
v) Recovery	:	MNPs	RF field	Recovery	EB	CP

EB: Intravenous injection of 0.3 μL 2% Evans Blue dye (30 min circulation time).
CP: Cardiac perfusion of 0.9% saline and fixation solution.
H_2O-dd: Injection of the 60 μL distilled water via catheterization into the left MCA.
RF field: Application of magnetic heating, exposure of the animal to the RF field (30 min).
MNPs: Injection of the 60 μL PMO-coated MNPs via catheterization into the left MCA.
Recovery: Two-hour recovery period during which the animal is anesthetized.

2.4. MNP localization using magnetic resonance imaging

Images of the selected brain samples were acquired by a 7 Tesla magnetic resonance scanner (Agilent Technologies). The samples were securely placed inside a custom-made coil with 2 channels and positioned in the middle of the magnet. To identify the MNPs in the brain samples, contrast-enhanced T2 spin-echo sequences were acquired using the following parameters: repetition/echo time, 3000 ms/20 ms; slice thickness, 1.00 mm; matrix size, 512 × 512; and pixel spacing (in plane resolution), 0.0488 mm^2.

2.5. Analysis of BBB opening using Evans Blue

Three brains from each group were sectioned coronally into 200 μm slices, from rostral to caudal on a vibratome (Leica, Germany) and collected on gelatin-coated glass slides. Sections were then mounted using a mounting medium and the EB staining was directly visualized and imaged with an epifluorescent microscope (Leica DM2000, Germany). The EB containing samples were imaged at 1× in phase-contrast mode with a 9 ms exposure time. Images were exported for luminosity analysis in tagged image file format (TIFF).