Blood-brain-barrier disruption dictates nanoparticle accumulation following brain injury

Vimala N. Bharadwaj[1], Rachel K. Rowe[3], Jordan Harrison[3], Chen Wu[3], Trent R. Anderson[3], Jonathan Lifshitz[2,3], P. David Adelson[2], Vikram D. Kodibagkar[1], Sarah E. Stabenfeldt[1]

[1]School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ

[2]Barrow Neurological Institute at Phoenix Children's Hospital, Phoenix, AZ

[3]University of Arizona, College of Medicine-Phoenix, Phoenix, AZ

Statement of Purpose: Traumatic brain injury (TBI) constitutes a significant public health concern at all ages[1]. Broadly, TBI can be classified into focal injury, associated with cerebral contusion and hematoma[2], and diffuse brain injury, a more subtle scattered microscopic pathology[2]. The complex progression of TBI may result in transient blood-brain-barrier (BBB) breakdown, contributing to leaky vasculature[3] that may ultimately provide a 'window of opportunity' to deliver nanoparticles (NPs) decorated with diagnostic and/or therapeutic agents. In laboratory settings animal models of brain trauma have been used to gain insights into the events that occur during and after injury. Due to the heterogeneity of the disease, any one animal model may not be able to fully recapitulate all the facets of injury pathology that are observed in human TBI[4]. Previously, we and others demonstrated the feasibility of delivering NPs to lesioned brain after a focal brain injury via the disrupted BBB[5,6,7]. However, the utility of NPs in other injury phenotypes such as diffuse brain injury was largely unknown. Therefore, we assessed NP-size dependent accumulation in two different diffuse brain injury models: fluid percussion injury and repetitive mild TBI. We hypothesized that similar to focal injury NP accumulation in diffuse injury models would correlate directly with vascular permeability.

Methods: Carboxylated polystyrene NPs of different sizes (20nm, 40nm and 500nm) were PEGylated to improve the blood circulation time[5]. Each of the NPs had unique fluorescent dyes and enabled simultaneous imaging with minimal overlap. All animal studies were approved by Arizona State University's Institute of Animal Use and Care Committee (IACUC) and were performed in accordance with the relevant guidelines. Two brain injury models were employed: fluid percussion injury (FPI) and repetitive mild TBI (rmTBI). The FPI animals received fluid percussion injury centered above mid-line and between bregma and lambda[8] (n = 3). While the rmTBI animals[9] were subjected to weight drop vertically to the top of an intact mouse skull. Animals were subjected to either one impact (100g weight) or one impact (50g weight) per day for five consecutive days (n = 3). The sham cohorts underwent the same surgical procedure as the injured group without receiving the injury. NP cocktail (13.3mg/ml of each NP) was injected retro-orbitally 2h post-injury and horseradish peroxidase (HRP) was injected 10mins before sacrifice. Animals were transcardially perfused 3h post-injury and the brain was harvested, cryopreserved, sectioned, stained (for HRP) and imaged with a slide scanner (HRP) or confocal microscopy (NPs).

Results: At 3h post-injury, FPI animals exhibited HRP extravasation into the brain parenchyma diffusely throughout the brain evidenced by dispersed HRP staining (Figure 1). Quantification of HRP staining revealed significantly higher HRP levels for FPI animals as compared to shams. Whole brain section confocal scans revealed NP accumulation co-localized with the HRP stained areas. Moreover, the quantification of the total intensity of each NP group (20nm, 40nm, and 500nm) showed significant increase in the injured brain compared to that of the sham brains 3h post-FPI (Fig. 1). In contrast to the FPI study, no HRP accumulation/extravasation was observed in the rmTBI group after either a single or repetitive impact(s). Similarly, NP accumulation was not observed in the rmTBI groups.

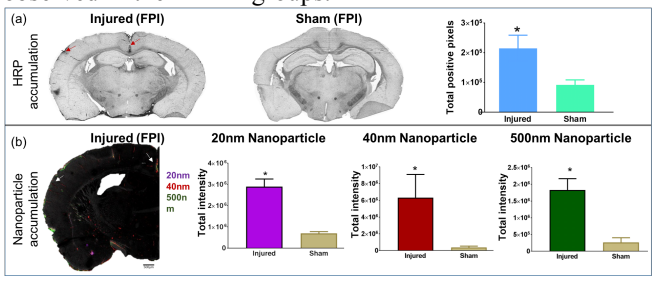

Figure 1. Nanoparticle accumulation 3h post fluid-percussion injury (FPI). (a) Injured (FPI) animals showed dispersed HRP compared to sham. (b) Confocal image of different size NPs (overlay with DIC). Arrows show accumulation of HRP (red) and NPs (white). *p<0.05 compared to respective sham groups, Student's t-test. Error bars represent standard error of mean, n=3 per group.

Conclusions: Passive systemic NP delivery to the injured brain hinges on a damaged, leaky BBB. In this study, we confirmed that the moderate diffuse FPI model resulted in a dysfunctional BBB as evidenced by HRP extravasation. Subsequently, PEGylated polystyrene NPs of up to 500 nm accumulated in the brain within HRP positive regions. In contrast, no NP/HRP accumulation was observed in the rmTBI groups indicating minimal to no breach of BBB and no mechanism for passive NP accumulation. The results of this study support our previous report with a focal brain injury model, where we demonstrated a direct correlation with HRP accumulation and NPs within the injury lesion[5]. These results establish the utility and potential for NP-based therapeutic and imaging tools for TBI in which the BBB is compromised.

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