Effect of PEG Modification on the Transport of Epidermal Growth Factor in the Stroke-Injured Brain

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Statement of Purpose: Stroke is a neurological disorder that currently has no cure. One strategy for treating stroke relies on growth factor-dependent neural regeneration after injury. Recombinant human epidermal growth factor (rhEGF) stimulates endogenous neural precursor cells in the subventricular zone (SVZ) leading to tissue regeneration in animal models of stroke[1]. A minimally invasive local delivery strategy is to deliver rhEGF from the brain cortex; however, this requires the protein to diffuse through the brain, from the site of delivery to the SVZ. Protein diffusion in the brain is limited by rapid removal from the extracellular space[2] and hence increased diffusion distance is necessary to ensure successful clinical translation. Here we aim to improve rhEGF penetration in both uninjured and stroke-injured mouse brains by modification with poly(ethylene glycol) (PEG).

Methods: We conjugated rhEGF to 5 kDa PEG. The number of PEGs per EGF was controlled by the functional group on PEG (NHS- and aldehyde terminated PEG were used), reaction pH and molar excess of PEG to rhEGF. Using integrative optical imaging (IOI) we examined the *ex vivo* diffusion of fluorescently labeled EGF and PEG_x-rhEGF (x = 1, 2, 3) in brain tissue slices harvested from both uninjured and stroke-injured C57/BL6 mice (8 – 10 wks old). The diffusivity (D) and elimination constant (k_e) were determined from all tissue types using mathematical modeling, and the resulting values were used to estimate the penetration distance of rhEGF and PEG*x*-rhEGF in brain tissue under both point-source delivery and constant-source delivery assumptions[3]. We then validated the *ex vivo* data with *in vivo* results. rhEGF and PEG_1-rhEGF were delivered either as a single injection or through a polymeric drug reservoir to the cortical tissue of uninjured mice. 50µm dorsal-ventral tissue sections were prepared and a rhEGF ELISA was performed to analyze the concentration of rhEGF or PEG_1-rhEGF in each section.

Results: By varying the pH and reactant molar concentrations, we obtained three formulations of PEG_x-rhEGF where x is 1, 2 or 3. IOI results indicated that PEG_1-EGF had the optimal balance between diffusivity (D) and rate of elimination (k_e) compared to rhEGF, PEG_2- and PEG_3-rhEGF, suggesting the potential for deepest tissue penetration[3]. Using both a point-source model and a constant-source model, the predicted tissue penetration distance of PEG_1-rhEGF is 2 – 3 times higher than rhEGF. Additionally, following release from a constant source, such as a polymeric scaffold, the penetration distances achieved by both rhEGF and PEG_1-rhEGF are calculated to be significantly greater compared to a point-source, such as a bolus injection[3].

The *ex vivo* modeling results were validated *in vivo* following both bolus delivery and delivery from a polymeric scaffold in uninjured animals. In both cases, the concentration of PEG_1-rhEGF was at least two-fold higher than rhEGF at the deeper tissue sections ranging from 2000 – 3000 µm (Figure 1). This was observed at 4 – 24 h following bolus injection and 6 h – 2 days following scaffold delivery, suggesting that PEG_1-rhEGF diffuses significantly further compared to rhEGF.

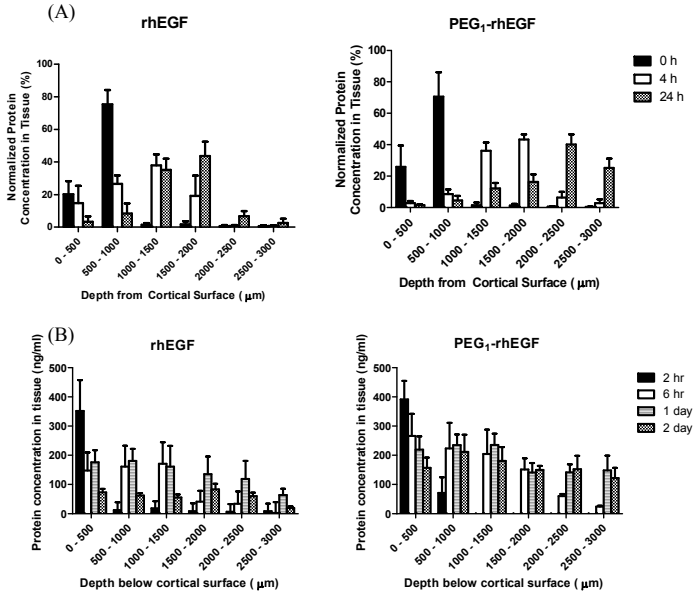

Figure 1. *In vivo* penetration distance of rhEGF and PEG-rhEGF in the uninjured mouse brain following (A) bolus delivery and (B) delivery from scaffold.

Discussion: The blood-brain barrier makes the design of a minimally-invasive delivery strategy more complex. By delivering therapeutically relevant proteins directly to the brain tissue, the BBB is circumvented. To overcome the diffusional limitation of proteins in brain tissue, we modified rhEGF with PEG. By monitoring the penetration distances both *ex vivo* and *in vivo*, we quantitatively show that PEG modification reduces the rate of rhEGF elimination by over an order of magnitude. This corresponds to a two to threefold increase in predicted brain penetration distance. Our findings suggest that a controlled release, minimally-invasive drug delivery system can be used to deliver therapeutically-relevant molecules directly to the brain and thereby promote repair.

We acknowledge funding from NSERC and the Heart & Stroke Foundation of Ontario

References: 1. Kolb B. J Cereb Blood Flow & Metab (2007) 27, 983 – 997. 2. Thorne R. J Neurophys, 92 (2004) 3471-3481. 3. Wang Y. J Control Rel (2010) doi10.1016/j.jconrel.2010.10.022