SUPPORTING INFORMATION

Therapeutic benefits from Nanoparticles: The potential significance of nanoscience in diseases to diseases with compromise to the blood brain barrier

Silke Krol[1], Richard Macrez[2,3], Fabian Docagne[2], Gilles Defer[2,3], Sophie Laurent[4], Masoud Rahman[5], Mohammad J. Hajipour[5], Patrick G Kehoe[6*], and Morteza Mahmoudi[5,7,8*]

[1] Fondazione IRCCS Institute of Neurology "Carlo Besta", Milan, Italy

[2] INSERM U919, Serine Proteases and Pathophysiology of the Neurovascular Unit. UMR CNRS 6232 Ci-NAPs, Cyceron, Université de Caen Basse-Normandie, Caen, France.

[3]Department of Neurology, University hospital of Caen, Caen, France.

[4]Department of General, Organic, and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, University of Mons, Avenue Maistriau, 19, B-7000 Mons, Belgium

[5]Laboratory of NanoBio Interactions (www.biospion.com), Department of Nanotechnology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

[6]Dementia Research Group, School of Clinical Sciences, Faculty of Medicine and Dentistry, University of Bristol, John James Laboratories, Frenchay Hospital, Bristol, UK

[7]Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

[8]School of Chemical Sciences, University of Illinois at Urbana-Champaign, 600 South Mathews Avenue, Urbana, Illinois 61801

*Corresponding Author: (P.K.) Email: Patrick.Kehoe@bristol.ac.uk; (M.M.) Web: www.biospion.com; Email: Mahmoudi@Illinois.edu.com

Pathways of brain infection

A comprehensive understanding of the mechanism of CNS infection is necessary for drug development against CNS-based pathogens. CNS infection is one of the most important causes of mortality and morbidity in the world - bacterial meningitis as one example is one of the top ten causes of infection-related morality in the word.[1] Importantly, several pathogens are able to pass through the BBB and infect the CNS. Many of these pathogens have a common mode for infection but the mechanism by which they passage through the BBB remains unclear. Pathogens may cross the BBB para-cellulary, via infected phagocytes (e.g. Trojan Horse) or transcellulary (See Figure S1).[2] In para-cellular entry, the permeability of BBB is increased due to increased pinocytotic activity or opening of tight junction and the formation of transendothelial channels.[3] Para-cellular penetration of the BBB is suggested for Teponema pallidum, Borrelia burgdorferi and Trypanosoma sp. [4, 5, 6, 7] (See Figure S1). Human and simian immunodeficiency viruses (i.e. HIV and SIV), L. monocytogenes, M.tuberculosis invade the CNS via the infected phagocyte route, in this case leucocytes [8, 9, 10]. In this (Trojan horse) pathway, these pathogens infect circulating monocytes, from which perivascular macrophage/microglia are derived, then the infected cells carry the pathogen through the BBB. Transcellular penetration of the BBB has been demonstrated for Mycobacterium tuberculosis [11], groupB *Streptococcus* [12], *E. coli* [13], *Cryptococcus* neoformans [14], Listeria monocytogenes [15], fungal pathogens such as *Candida albicans* [16] and suggested for the West Nile virus (WNV) [17]. The transcellular route usually involves more than 90% of bacteria binding to surface microvili but this association has no significant membrane changes (See Figure S1).

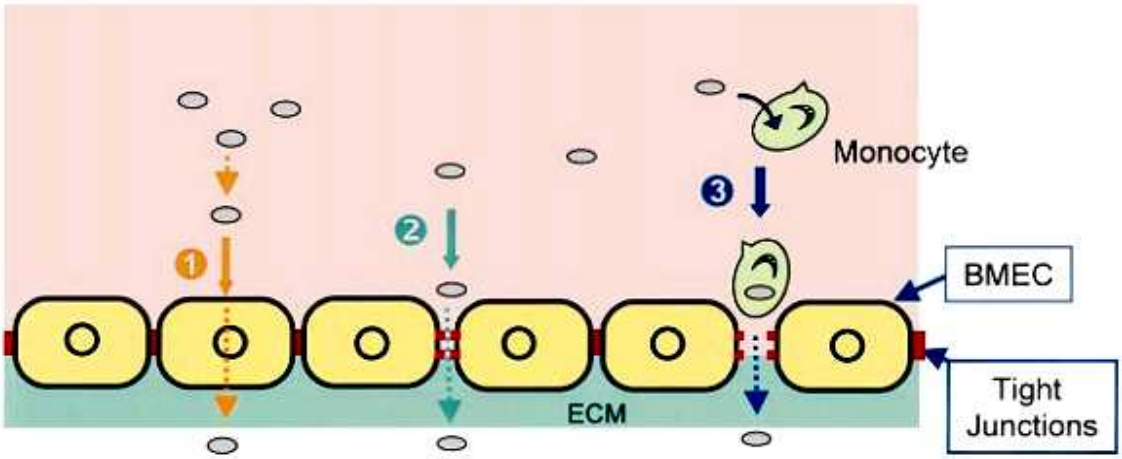

Figure S1: The mechanisms of microbial passage through the BBB. 1. Transcellular pathway, 2. Paracellular pathway, 3. Trojan horse pathway. (Reproduced with permission from John Wiley and Sons)

Microbial invasion factors and cell receptors involved in invasion mediate the endothelial interaction

Invasion and binding of microbial pathogens to host cell receptors are key steps involved in the crossing of the BBB. Therefore ligand-receptor interactions are likely relevant in CNS infection. Group *B Streptococcus*, *E. coli*, and *S. pneumoniae* can achieve levels of bacteria in the blood (i.e. bacteremia) which are needed for invasion and penetration through the BBB.[13] These bacteria are associated with inflammation of the meninges (i.e. meningitis) through rearrangements of the actin cytoskeleton. Although it has been shown that high levels of becteremia are necessary, but not sufficient to ensure penetration across the BBB. A comprehensive understanding of the mechanisms involved in ligand-receptor interactions help us

to develop drugs that block these interactions and in turn CNS infection. Cytotoxic necrotizing factor1 (CNF1) and Ibe Proteins of *E. Coli* contribute to BMEC invasion by ligand–receptor interactions. In fact CNF1 binds to 37-kDa laminin receptor precursor and is in turn internalized by receptor-mediated endocytosis.[2] The outer membrane protein, OspC, of *Neisseria meningitides* attaches to fibronectin and the resultant complex then binds to the integrin a5b1 receptor on the human BMEC surface to mediate internalization of this bacterium.[18] Invasion of *Listeria monocytogenes* and *S. pneumoniae* through the BBB is mediated by internalin B and CbpA, respectively.[15] The viral coat glycoprotein gp120 of HIV binds to the CD4 receptor on host cells and mediates its internalization into the cells. There is no CD4 receptor on neurons; therefore this is not possible for HIV to infect them by binding to the surface receptors. Instead HIV binds to CD4 receptors on the immune cells where in turn the infected cells can cross the BBB (Trojan horse transport).[19,20] Glycoseaminoglycans (GAGs)-binding proteins of microbes may be used for adhesion purposes which mediate the binding and passage of pathogens through the BBB. The surface of mammalian endothelial cells is coated with GAGs that have negatively charged sulphates.[21] Heparan sulphate is expressed on the surface all of eukaryotic cells which bind to microbial GAG-binding protein, growth factor and apolipoprotein.[22] Chang and co-workers[23] demonstrated that the interaction between GAGs (Heparan sulfate) and the leading meningitis pathogen group *B Streptococcus* (GBS) is very important in how it penetrates the CNS. They showed that site-directed mutagenesis of a GAG-binding domain of the surface of GBS alpha C protein and/or genetic impairment of GAG expression, decreased GBS penetration into the brain. Therefore GAGs and GAG-binding proteins pose as suitable targets for new drugs to suppress the CNS infection.

Nanotechnology as a new device for delivery of antimicrobial drugs to the brain, challenges and opportunity

BBB and BCSF are two main obstacles that work to prevent access of exogenous compounds to the brain. BBB is the primary barrier at the interface between CNS and peripheral circulation.[24] BCSF is a secondary barrier between choroid plexus endothelial cells and tight junctions.[25] The passage of molecules (e.g. drugs) through these barriers is dependent on physicochemical properties such as size, degree of ionization and lipophilicity. Drug delivery to the brain using drug carriers of nanometer scale offer a promising strategy to overcome physiologic barriers and help guide drugs to their targets. The small size, flexibility and versatility of nanoparticles make this very viable. Nanocarriers can be engineered by the attachment of specific ligands to achieve better biodistribution, biocompatibility and pharmacokinetics to improve the efficacy of treatments for CNS infection.[26] Nanocarriers can also carry the biological drugs, protect them from degradation whilst delivering the drugs to specific sites.[27] Nanocarrier systems which include polymer/denrimer, liposoms, micelles and nanoparticles, help to deliver agents through the BBB.[28] Several nanocarriers such as Poly (butyl cyanoacryalate) (PBCA) [29], poly (D,L-lactide-co-glycolide) (PLGA), poly-lactide (PLA)[30], albumin, chitosan, phospholipids and pluronic p85 have also been used to similar effect. These nanoparticles were modified with compounds such as Polysorbate 80[31], poly ethylene glycol (PEG)[32], apolipoprotein E[33],

monoclonal antibody (mAb)[34], Tat[35] and transferrin[36] to enhance their BBB permeability via Receptor-mediated transcytosis and Adsorptive-mediated transcytosis methods. Xu *et al.*[37] reported that amphotericin B-poly butyl cyanoacrylate nanoparticles (AmB-PBCA-NPs) modifed with polysorbate 80 carried amphotericin B (as a classic antifungal drug) through the BBB and is promising treatment against cryptococcal meningitis in a mouse model. Pandey and Khuller also showed that orally administered poly-lactide-co-glycolide nanoparticles which encapsulated antituberculosis drugs (ATDs) (rifampicin+isoniazid+pyrazinamide+ethambutol) transported the ATDs into brain and are effective against Mycobacterium tuberculosis.[38] The penetration of anti-retroviral drugs through the BBB is highly critical for the treatment of HIV-associated CNS complications. Recently Mahajan *et al.*[39] demonstrated that the antiviral drug, Saquinavir, stably incorporated within Transferrin conjugated quantum rods crossed the BBB and decreased the HIV-1 viral replication. This new nanoformulation poses as a promising candidate for the treatment of Neuro-AIDS and other neurological pathogenesis. Research into the delivery of other anti-retroviral drugs is also ongoing. Rao *et al.*[40] reported that nanoparticles conjugated to TAT peptide which encapsulated ritonavir, a protease inhibitor (PI), crossed the BBB and bypassed the efflux action of P-glycoprotein and effectively decreased viral load (HIV) in the CNS. A new strategy for the treatment of multiple-drug resistant infections involves the use of cationic antimicrobial peptides. Wang *et al.*[41] indicated that cholesterol-conjugated G_3R_6TAT (CG3R6TAT) which has antimicrobial activity, penetrated the CNS and suppressed the growth of *Cryptococcus neoformans* (yeast-induced brain infections). To get antibiotics into the CNS, Liu *et al.*[42] designed biologically active polymer core/shell nanoparticles (i.e.micelles) self-assembled from TAT-poly(ethylene glycol) (PEG)-b-cholesterol (TAT-PEG-b-Chol). These nanoparticles were used to transport Ciprofloxacin (as a model antibiotic) through the BBB

which they did successfully. So far nanoparticles have been mostly used as nanocarriers of antimicrobial drugs such as antibiotics for treatment of CNS infection. However, the increasing occurence of antibiotic- and/or multidrug-resistant pathogens suggests the urgent need to have new strategies to be able to make centrally available these biocompatible nanoparticles that have natural antibacterial activity. Recently, we designed a new multifunctional silver-gold coated superparamagnetic iron oxide nanoparticles (SPIONs) that can be potentially used as multimodal antibacterial agents since they have strong antibacterial activity against multidrug resistant *Staphylococcus aureus* and *Staphylococcus epidermis*. These nanoparticles are also fully compatible with most human cells and are promising candidates to use against CNS pathogens, although they still require some modifications to allow them to cross the BBB.[43]

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