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Quantification of kinetic rate constants for transcytosis of polymeric nanoparticle through blood-brain barrier

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ABSTRACT

Background: Polymeric nanoparticles (PNP) have received significant amount of interests for targeted drug delivery across the blood-brain barrier (BBB). Experimental studies have revealed that PNP can transport drug molecules from microvascular blood vessels to brain parenchyma in an efficient and non-invasive way. Despite that, very little attention has been paid to theoretically quantify the transport of such nanoparticles across BBB.
Methods: In this study, for the first time, we developed a mathematical model for PNP transport through BBB endothelial cells. The mathematical model is developed based on mass-action laws, where kinetic rate parameters are determined by an artificial neural network (ANN) model using experimental data from *in-vitro* BBB experiments.
Results: The presented ANN model provides a much simpler way to solve the parameter estimation problem by avoiding integration scheme for ordinary differential equations associated with the mass-action laws. Furthermore, this method can efficiently deal with both small and large data set and can approximate highly nonlinear functions. Our results show that the mass-action model, constructed with ANN based rate parameters, can successfully predict the characteristics of the polymeric nanoparticle transport across the BBB.
Conclusions: Our model results indicate that exocytosis of nanoparticles is seven fold slower to endocytosis suggesting that future studies should focus on enhancing the exocytosis process.
General significance: This mathematical study will assist in designing new drug carriers to overcome the drug delivery problems in brain. Furthermore, we anticipate that this model will form the basis of future comprehensive models for drug transport across BBB.

1. Introduction

The blood-brain barrier (BBB) is a distinct and highly selective barricade formed by the microvascular endothelial cells together with the other neurovascular elements such as astrocytes, pericytes, and microglial cells [1,2] to protect the brain against harmful agents. The endothelial cells in the BBB are connected to each other through tight junctions. These tight junctions preclude paracellular transport of solutes across BBB except some water-soluble substances. Several transcellular routes, such as transporter protein mediated, receptor-mediated, adsorptive mediated, cell-mediated transcytosis *etc.* are available for transportation of metabolites and other essential components for proper functioning of brains [3]. However, these transport routes cannot be used for delivering large-molecule drugs and > 98% of small-molecule drugs across the BBB [4]. To enhance the efficacy of therapeutic delivery through the BBB, several techniques have been tested with varying degrees of success. Among various techniques, tight junction disruption, drug molecules modification and carrier-mediated transportation have dominated the drug delivery research.

Tight junction opening is reported using biological, chemical, and physical stimuli [3]. Biologics such as virus, macrophage, cereport, zonula occludens toxin *etc.* can increase paracellular transport by either opening tight junctions or using Trojan horse effect [3]. Selected chemicals such as cyclodextrin and poloxamers can extract water and other substances (*e.g.* cholesterol) from endothelial cells, which lead to opening of gaps between cells for paracellular transport. Physical mechanisms such as ultrasound, microwave, and electromagnetic field can also open tight junctions by protein translocation, which enhance the BBB permeability. Although various stimuli can potentially increase the penetration of drugs to the brain, high concentration of these stimuli compounds can compromise the BBB.

Drug delivery across BBB through modification of drugs is attempted either by direct conjugation of drugs to a BBB transporter (such as glucose transporter) or by targeting lipid-mediated transport of

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drugs. However, because of highly selective nature of transporters, drugs transport by direct conjugation to a transporter is only possible if drug molecules meet all criteria of endogenous ligands [5]. Lipidization is done by attaching lipid-like molecules on the drug structure by modifying hydrophilic moieties. Although high lipophilicity favored higher permeability of drugs, this lipidization does not ensure targeted drug delivery because the permeability of lipidized-drugs increases across all biological membranes in the body [6]. Moreover, this approach is only suitable for drugs having molecular weight < 500 Da since higher molecular weight compounds cannot cross BBB through passive lipid soluble mechanism.

Another promising avenue for drug transport across BBB is through carriers such as liposomes, nanoparticles, nanospheres, nanosuspensions, polymer micelles, and nanogels [7]. Among them, nanoparticle-based therapeutic delivery is extensively studied because of their non-invasiveness and targeted drug delivery capability [8]. As a results, several nanoparticle-based system such as mesoporous silica [9,10], silver [11], superparamagnetic iron oxide [12], gold [13], polymeric [14] nanoparticle have been developed and tested as a drug delivery mechanism through BBB with different levels of success. Polymeric nanoparticles offer several advantages over non-polymeric ones because of their similarity with natural carriers such as serum lipoproteins and viruses. Moreover, they can be targeted not only to the particular organ/tissue, but also to a particular cell or even an intracellular compartment [7]. In addition, polymeric nanoparticles increase drug solubility, improve bio-distribution of drugs, and can potentially decrease side effects [14].

Although significant efforts have been made to study the nanoparticle transport through BBB experimentally, not much attention has been paid to theoretically quantify the transport mechanism. Mathematical modeling is a powerful tool to understand the biological processes such as transport across BBB, where experiments are intricate, costly and sometimes ethically wrong. Hinow et al. [15] developed a mathematical model to describe the transport of free drugs from blood to brain once drugs get released from liposomes under the application of focused ultrasound. However, their model neglects drug accumulation by the endothelial cell and active efflux of drugs from brain to blood. The quantification of accumulated drugs is very important because accrual of particles can increase the toxicity in endothelial cells which may alter the BBB integrity. Loeser [16] modeled the drug release from a temperature-sensitive liposome and the diffusion of drugs in a brain cancer cell, but their model does not include the transport of liposomes from blood to targeted brain cancer cells. Recently, Khan et al. [17] developed a mathematical model for receptor-mediated transcytosis of iron across BBB. Although our previous model addresses the transcytosis, it can't be used to predict the nano-carrier based drug transport across the BBB because of difference in mechanisms and pathways between iron molecule and nanoparticles.

The goal of the current research is to develop a mathematical model for nanoparticle transport across the BBB using the laws of mass-action. Mass action laws can generally be formed as a set of ordinary or partial differential equations. The estimation of parameters is the crucial step in the development of these type of models. Several methods are developed and used for the parameter estimation such as least square [18], Bayesian approach [19], incremental approach [20], artificial neural network approach [21], preprocessing method [22] *etc.* Among these methods, least square is the oldest, simplest and widely used for parameter estimation. However, this method suffers from convergence problems. In addition, this approach may get trapped into the local optimal solution instead of the global one [21,23]. In this work, an artificial neural network (ANN) based model is presented for parameter estimation. Our ANN based parameter estimation method is much simpler to solve since it does not require an integration scheme for differential Eqs. [21]. In addition, ANN can deal with large data sets, can efficiently approximate highly nonlinear functions, and can be used for multi input-output variables [24]. To train our ANN model, we have considered the transcytosis of poly[Triphenylamine-4-vinyl-(P-methoxy-benzene)] (TEB)-based nanoparticles through BBB. We specifically selected TEB-polymeric nanoparticles because TEB nanoparticles exhibit excellent fluorescence properties which eliminates the necessity of tagging with additional fluorescence markers. Thus, this type of nanoparticles can potentially be used in both imaging and drug delivery. Moreover, TEB nanoparticles can be synthesized as small as 20 nm. It has been reported that smaller size (~20 nm) nanoparticles yield higher transcytosis across BBB [13,25,26]. In addition, the TEB nanoparticles are highly biocompatible. Controlled transcytosis experiments of TEB nanoparticles across BBB are performed on an *in vitro* BBB model which is constructed based on mouse cerebral endothelial cells (bEnd.3).

Rest of the paper is organized as follows. In the following section, mathematical model for nanoparticle transport through BBB endothelial cells is presented. Next, an ANN model is presented for estimation of kinetic parameters. In section 4, we describe the *in-vitro* experiments of both endocytosis and exocytosis to determine the nanoparticle transport rate across the BBB. In section 5, we present important results such as estimated rate parameters for nanoparticle transcytosis and time required for nano-carrier penetration. Finally, we present our conclusions and future research outlook.

2. Mathematical model

A mass-action based mathematical model is presented to study the transcytosis of nanoparticle from the upper (luminal) chamber to lower (abluminal) chamber as shown in Fig. 1. Endocytosis of nanoparticles can occur by various active transport mechanisms. At the same time, endocytosed nanoparticles may recycle back from cell to upper compartment due to the presence of active efflux pumps in BBB endothelium. The endocytosis and recycling of nanoparticles can be expressed with the following reversible kinetic equation.

$$N_{up} \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} N_{cl} \tag{1}$$

where N_{up} and N_{cl} represent the number of TEB nanoparticles in upper compartment and cell, respectively, k_1 and k_{-1} are the overall rate of endocytosis and recycling of nanoparticles, respectively. The fusion machinery of BBB endothelium transports the nanoparticles from cell to lower compartment of transwell through basolateral membrane by an exocytosis mechanism [27,28]. Like recycling process through the apical membrane in the luminal side, transported nanoparticles can be internalized from abluminal side (lower compartment) to endothelial cell through basolateral membrane. This process is termed as reendocytosis in the literature [29]. The exocytosis and reendocytosis of nanoparticles can be expressed by the following reversible first order kinetic equation.

$$N_{cl} \underset{k_{-2}}{\overset{k_2}{\leftrightarrow}} N_{lo} \tag{2}$$

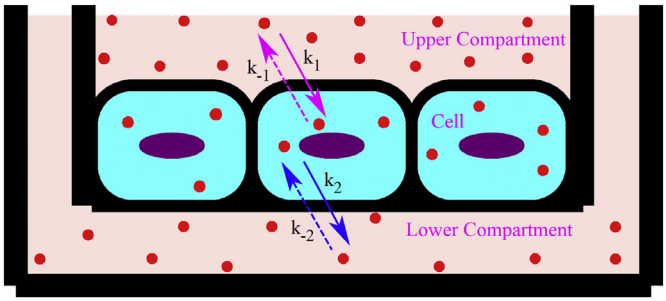

Fig. 1. Schematic of nanoparticle (filled circle) transcytosis through a transwell BBB model. At time $t = 0$, nanoparticles are introduced on the upper chamber for transcytosis.