Kinetics of Proteins in the Blood-Brain Barrier

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Abstract: The delivery of chemotherapy for cancer into the central nervous system, in particular the brain, remains a challenge. This results in brain metastases commonly being a cause of death from cancer. Here, we look at the environment of the blood-brain barrier. Then, we explore two proteins (breast cancer resistance protein and p-glycoprotein) that may inhibit the transport of medications (erlotinib and flavopiridol) across the blood-brain barrier. Next, we look at a mathematical model to quantify the effect of these two efflux-inducing proteins on transport. Last, I create a COMSOL model to describe and predict behavior at the blood-brain barrier (BBB) with respect to one of the chemotherapeutic agents (erlotinib).

Keywords: Cancer, Chemotherapy, Kinetics, Blood Brain Barrier.

1. Introduction

Cancer remains a leading cause of death in the United States and the world. In the U.S., it is the 2nd leading cause of death. 1.6 million people are diagnosed with cancer each year in the U.S., and 600,000 die [6]. Two common cancers are lung and hematologic.

New treatments have emerged based on genetic and molecular medicine, which have shown significant promise. For lung and blood cancer, these include erlotinib and flavopiridol. These are molecular based therapies that target and inhibit proteins involved in tumorogenesis, and the products of mutated genes.

Despite the promise of such new treatments, these treatments are not a cure. One reason remains that many cancers, including lung and blood, cause death by invading the central nervous system, thereby causing brain death. The brain has special barriers that prevent the transit of chemotherapy from the blood stream into the neural cells.

From a quantitative perspective, the active metabolite of erlotinib in the blood has as concentration around 90 ng/ml [9]. In the lung tissue, the concentration is 10 ng/ml. However, in brain tissue, it is on the order of only 1 ng/ml. This is only 1% of the concentration administered to the patient, and likely only 10% of what a tissue needs for full efficacy.

One mechanism of inhibition of transport by the blood-brain barrier is efflux proteins located in the membranes of endothelial cells that serve as a barrier between the blood vessel and the brain cells and tissue. Two of these proteins are p-glycoprotein (p-gp) and breast cancer resistance protein (bcrp). They cause efflux of the chemotherapy molecules from the endothelial cell by active transport. The study of the kinetics of these two proteins can quantify how much of an effect they have on chemotherapy delivery.

2. Materials and Methods

The contribution of bcrp and p-gy to the blood-brain barrier can be measured by determining the partition coefficient. The partition coefficient will give a comparable ratio of solute (chemotherapy) in the blood stream to that diffused into the cell. The ratios between coefficients of wild type and resistance protein knocked-out cell lines can give us a better quantitative comparison.

The partition coefficient can be calculated from the permeability surface area products (PS) as shown below. The ratio of permeability surface area products gives the net flux through a barrier, and therefore, the partition coefficient at steady state.

The permeability surface area product is the following dividend: A/t/Co (transported amount of compound per mg protein of cell monolayer/incubation time/concentration of compounds on the donor side).

PS = A/t/Co (amount transported/incubation time/concentration on donor side).

Then R, the partition coefficient ratios are as follows:

R(bcrp) = PS(bcrp knock-out)/ PS(wild type)

R(p-gp) = PS(p-gp knock-out)/ PS(wild type)

R (p-gp and bcrp knock-out) = PS(p-gp and bcrp knock-out)/ PS(wild type).

In prior study, bcrp and p-gy are both associated with net decreased influx of chemotherapy, erlotinib and flavopiridol, into the brain[1]. P-glycoprotein has an R of 2.95, and bcrp of 1.29, for erlotinib. The results for flavopiridol were similar at 3.49 for p-gy, and 1.27 for bcrp. Double-knockouts have R values of 8.53 and 14.2, respectively. Both proteins significantly hinder the transport of chemotherapy into the diseased cell (Table 1).

Variable	Erlotinib	Flavopiridol
Wild-type	1.00	1.00
Bcrp knockout	1.29	1.27
P-gy knockout	2.95	3.49
Double-Knockout	8.52	14.2

Table 1. Partition Coefficient ratios (K_p) as determined in a mouse model [1].

3. Use of COMSOL Multiphysics® Software

We developed a COMSOL Model to model the distribution of concentration as a function of time, using an available template for partition coefficient analysis.

There are three layers of transport for the delivery of chemotherapy via the blood to brain tissue. First, the pharmaceutical crosses the basal side of the endothelial cell residing on the capillary. This occurs by transmembrane diffusion. Next, the molecule crosses the endothelial cell by general diffusion. Then, the drug crosses the boundary between the endothelial cell and the astrocyte (or neuron), by transmembrane diffusion again.

We used a dimensionless model. This allowed the creation of a mesh and plot that illustrates concentration in the neuron as a function of time, with relationship to the relative change in the partition coefficient.

We used the partition coefficient to create a linearly dependent flux across the two boundaries (blood vessel-epithelial cell, and epithelial cell-neuron). As follows:

Kp = C1final/C2final
Inward Flux to C1 = Constant * (C1 – Kp*C2) (mol/m2 * s)

We divided the epithelial cell into two boundaries and assumed that the flux was equal on both sides with respect to concentration.

We used three geometric- rectangles of equal size to model (the width of the capillary is nearly the same as the width or diameter of the epithelial cell and main process of an astrocyte or neuron - on the order of 10 micrometers). For the general diffusivity, we used common parameters (the general diffusivity in a cell, from healthy to cancerous ranges on the order of 10^{-12} m2/sec). Also, we used a standard starting concentration. We then created a plot using the R value ratios for erlotinib, with time as the progressing variable.

4. Figures

Dimensionless plots of erlotinib distribution as function of time with wild-type and double-knockout genotypes. An arbitrary starting concentration of .125 was used. The top geometric-rectangle in each figure represents the neuron, the middle geometry the endothelial cell, the bottom the blood vessel.

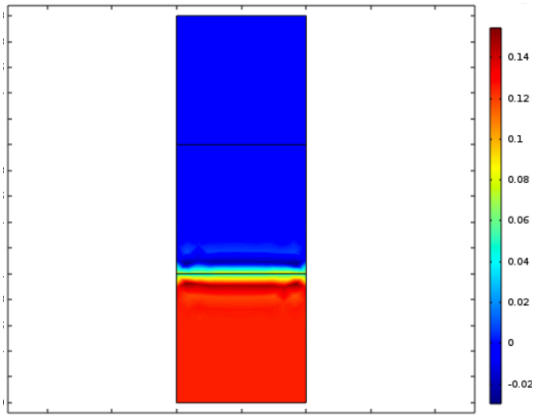

Figure 1. Wild-type, t=0

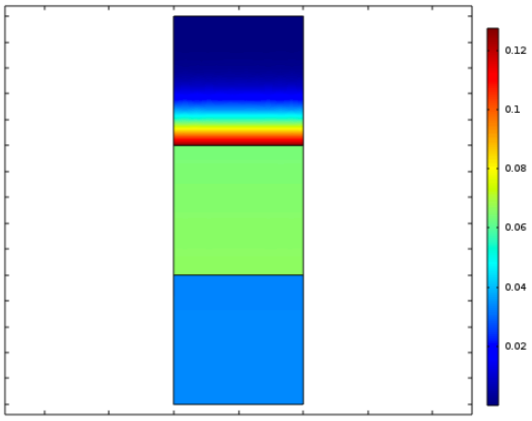

Figure 2. Wild-type, t=1

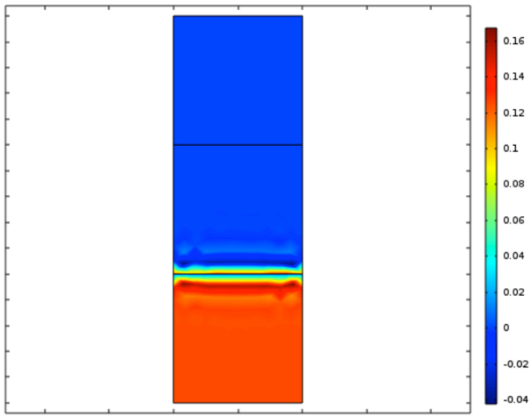

Figure 3. Double-knockout, t=0

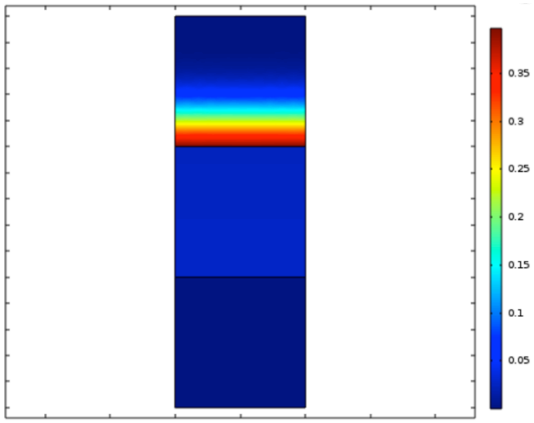

Figure 4. Double-knockout, t=1

5. Conclusion

COMSOL is effective to plot the diffusion of erlotinib as a function of time and partition coefficient. COMSOL can likely be used in the future to better depict and illustrate concentrations to facilitate more detailed models. Of note, the COMSOL models do depict that the epithelial cell reaches a steady state distribution - a uniform concentration. This would support not including it in models, as when the time increases the amount of chemotherapy delivered to the neuron or astrocyte is a reflection of the concentration in the blood and overall partition co-efficient. Nonetheless, it is useful to create a model including the epithelial cell as it is the key factor in the blood-brain barrier.

6. Discussion

We studied the kinetics of pharmacologic blood-based therapy for cancer, with respect to resistance at the blood-brain barrier to entry. Mathematical analyses can be used to delineate the relationship of proteins involved in efflux and resistance to entry at the blood-brain barrier. This is via measuring permeability solubility products and partition coefficients, and then using mathematical projections to compare with experimental data. Furthermore, membrane kinetic analyses may be used to develop a computer-aided design that can further elucidate the distribution of concentration of chemotherapy through all layers of transport.

In the future, this kinetic study may be used to further investigate these efflux proteins and the transport of new chemotherapeutic agents.

7. References

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