Application of in vitro BBB model to measure permeability of nanoparticles

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Abstract. In both pharmaceutical and toxicological fields, one of major issues has been the possibility of nanoparticle uptake to central nerve system. For the safe use of nanoparticles, it is integral to evaluate the permeability of nanoparticles through BBB. In our collaborative research group reported that a few nanoparticles accumulated in brain in animal experiment, as an in vitro model, we applied commercially available cell-based BBB model for establishing evaluation method, which is quick, quantitative and equivalent to in vivo assay. We assayed 30-1500 nm silica and surface charge dependent Qdots. Our results showed the size-dependency and the surface modification dependency. We compared our assay to several animal experiments. There are both equivalence and discrepancy with animal experiments. Our BBB model can be useful tools for evaluating size-dependent permeability, but not for surface modification-dependent permeability. Our BBB assay is non-serum assay and we have not adequately reflected the serum-related interaction between nanoparticles and cell surfaces. To clear up the discrepancy of our BBB model, serum-based assay and low-concentration detection will be needed.

1. Introduction

Nanoparticles (NPs) have great functionalities and potentials in many industrial fields. In also a bio-medical field, these are benefits of NPs for contributing to various novel medicines. For example, NPs are potential carriers of drug delivery systems for treatment [1-2] and some fluorescent, magnetic or radiolabelled NPs are useful as imaging materials for diagnosis [3-5]. In contrast, researchers are concerned about NPs give any stress to biological systems. Not a few research groups reported the risk. For instance, NPs in sun cream can stress brain cells [6], and NPs can induce brain edema formation by influencing BBB breakdown in vivo [7].

There are many parameters to evaluate the risk of NPs. NPs have many characteristics like size, materials (metal, oxide, semiconductor and organic), surfaces (charge and specific interaction) and shapes (round and sharp), and there are many exposure routes to the body, like oral, dermal, via pulmonary and via systemic circulation. The most important thing is that there are also many evaluation indexes such as inflammation, brain activities, NPs’ accumulation in brain and BBB
permeability. To evaluate all the possible risks, we need an evaluation method, which is quick, quantitative and equivalent to in vivo experiment (We would like to call “3Q evaluation method.

Our collaborative research group, previously, reported that, in animal experiment, surface-modified Qdots, which we know as one of the fluorescent NPs, are able to cross the BBB and remain in the brain parenchyma at 6 hour after intra-peritoneal (i.p.) administration [8]. In the research, 0.1% of NPs accumulated in brain by the i.p. injection in mice at 6hr while 8.8% of NPs stayed in blood circulation. In the histological analysis, red fluorescent Qdots accumulated in many areas of brain parenchyma, such as olfactory bulb, cerebral cortex, hippocampus, thalamus and brainstem. From this result, we know that, though the amount is few and small, NPs can be certainly in brain. We do not know so far where NPs transport into brain from exactly, but blood-brain barrier (BBB) will be one of solid pathways into brain.

Recently, we applied new cell-based in vitro BBB model to evaluate NPs permeability into brain, which is a commercially available culture system (PharmaCo-cell company, Japan), and is culturing both rat brain micro-vascular endothelial cells and pericytes separated by millicell membrane for mimicking the actual BBB [9]. The model has equivalence to in vivo experiment in pharmaceutical drug screening test. This assay procedure is as follows: (1) Reconstruction of the BBB model under astrocyte pre-cultivation, (2) Doing BBB permeability assay and measuring drug concentrations and (3) Calculating permeability coefficient (Papp). Papp represents apparent permeability of a total BBB membrane (both cell layers and a plastic membrane).

We can assay BBB permeability of NPs by using this BBB model. We evaluated 30-1500 nm silica and surface charge dependent Qdots by Papp. The Papp had size-threshold between 30- and 100 nm. Cationic surface-modified NPs can pass through the BBB model.

![Figure 1. The left image is a picture of actual BBB model. Endothelial cells form vascular walls and pericyte supported cell-cell contact of the endothelium. Astrocyte is also important for maintaining tight-junction of vascular cells. The right image shows the cell-based BBB model. Endothelial cells are at the top of the membrane, pericytes are at the bottom of the membrane, and before doing the assay, this model pre-cultures with astrocytes.](image)

2. Aim of this study
We compared between our cell-based BBB assay and animal experiments performed by other research group to confirm the equivalence of our in vitro assay to in vivo experiment.

3. Results-validation of the BBB model as 3Q evaluation method
This assay is a quick (within 30 min) and quantitative (usage of Papp) evaluation method. We validated the equivalence to animal experiment. Concerning the size-dependent permeability, our assay has size-threshold for BBB permeability between 30 and 100-nm; while, in animal experiment, a research group reported that 70-nm silica accumulate in the brain [10]. We should consider BBB permeability of nanoparticles with tens of nano-meter in diameter. Our assay can be qualitatively equivalent to in vivo experiment.
Concerning the quantitative relevance between our assay and animal experiment, we briefly calculated brain-blood amount ratio form the amount (weight/mole) of nanoparticles in the blood and the brain sides. The value in our in vitro assay is 0.018 in 30 min., while the value in previous animal experiment is 0.011 in 6 hr. Though the administration concentration in our research is much higher than that of the realistic exposure, these values are comparable to each other.

We have to report the limitation of the model. There is a discrepancy between our results and those of a previous in vivo study. In our study, the cationic nanoparticles passed through the BBB model more easily than the anionic and neutral nanoparticles. The reason for this result is that the cationic NPs strongly interacted with the negative charged cell surface.

So far, there are some shortcomings in our BBB model for evaluation of NPs transportation into brain as an alternative to animal experiments. For example, in animal experiment, anionic nanoparticles accumulated in brain more easily, in contrast to the result in our BBB assay. In our assay, we did not use serum in the assay buffer by following conventional protocols, but in the human body, NPs are thought to absorb serum proteins, which enhance cell-NPs interaction, like a "protein corona" in the Figure 2 [11]. Furthermore, in the human body, we have to consider the effect of NPs that accumulate in the blood vessels or in the endothelial cells. Further experiments will be needed to fill gaps between in vitro and in vivo experiments. In future, we will try serum-based assay or long time evaluation, and improve our model like a brain slice culture and a low-concentration detection method.

**Table 1.** Comparison between in vitro assay and in vivo experiment

| Experimental results   | In vitro                     | In vivo                      |
|------------------------|------------------------------|------------------------------|
| Size specificity       | Between 30- and 100-nm       | 70 nm                        |
| Surface modification   | Cationic > Anionic           | Anionic > Cationic           |
| Evaluation methods     |                              |                              |
| Quickness              | 30 min                       | 6 hr                         |
| Quantitativity         | Papp                         | Disposition in organs        |
| Equivalence            | To animal experiment         | Unknown to human             |

**Figure 2.** The image is illustration of cell surfaces and nanoparticles by forming “protein corona”
4. Conclusion

The in vitro BBB model can be “3Q” evaluation method of NPs’ permeability through BBB; we observed size-threshold for passable NPs into brain. Further improvement will be needed, like serum-based assays and low-concentration detection methods.

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