Optimizing transferrin-mediated transcytosis

By Kai-Jye Lou, Senior Writer

Researchers at the **California Institute of Technology** have shown how modulating the transferrin content on gold nanoparticles can optimize their delivery into the brain via the transferrin receptor.[1] The group now needs to determine whether its strategy will translate to drug-nanoparticle conjugates amenable for use in the CNS.

The normal function of the transferrin receptor is to engage with transferrin to bring the iron-binding protein into cells that express the receptor. In the endothelial cells that line the vasculature of the brain, the receptor helps shuttle transferrin from one side of the blood brain barrier (BBB) to the other—a process called receptor-mediated transcytosis.[2]

Prior efforts to exploit the transferrin receptor's mechanism to improve the delivery of therapeutic agents into the brain involved conjugating the agents to transferrin receptor–binding mAbs or transferrin-conjugated nanoparticles.[3–5] However, few such conjugates were successful.

A key breakthrough came in 2011 when researchers at **Roche**'s **Genentech Inc.** unit realized that antibodies with high affinity for the transferrin receptor were being trapped in the endothelium because they remained bound to the receptor.[6–8]

This led the Genentech group to develop lower-affinity mAbs that would be released from the receptor after crossing the BBB and accumulate in the brain at higher concentrations than high-affinity mAbs.

The Caltech group has now found that a similar principle applies to transferrin-conjugated nanoparticles.

The researchers synthesized a series of gold nanoparticles of different diameters and surface transferrin content. In mice, nanoparticles with diameters of 40–80 nm and moderate transferrin content bound to the transferrin receptor, penetrated the BBB and accumulated in the brain parenchyma.

In contrast, nanoparticles with low transferrin content showed weak binding to the transferrin receptor and failed to penetrate the BBB. Those with high transferrin content bound to the receptor but appeared to stick on or in endothelial cells (*see* **Figure 1, "Model of receptor-mediated transcytosis of transferrin-containing nanoparticles"**).

Results were reported in *Proceedings of the National Academy of Sciences*.

The researchers used gold nanoparticles in the model system because they are easy to modify and visualize. Similar gold nanoparticles are in clinical trials for non-CNS conditions, but they are not biodegradable and are unlikely to be suitable for clinical use in the CNS.

"We carried out this study because we wanted to translate the idea of modulating transferrin receptor binding in antibodies to optimizing a nanoparticle's ability to cross the BBB and access the brain," said Devin Wiley, lead author on the paper, who is now a medical student at the **Keck School of Medicine of the University of Southern California**. "We want to use the data generated in our study as a basis for understanding how to design other classes of nanoparticles that could be used in the therapeutic setting to target the brain, such as polymeric or liposomal nanoparticles."

"The data in this study, at a general level, suggest that the density of transferrin molecules on a nanoparticle can make a difference in how well it penetrates the BBB," said Pieter Gaillard, cofounder and CSO of **to-BBB technologies B.V.**

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>
> ***—Devin Wiley, Keck School of Medicine of the University of Southern California***

"This study confirms the results of earlier studies carried out by our group and others showing that transferrin-coated nanoparticles can help enhance the delivery of drugs into the brain," added Jörg Kreuter, a professor at the Institute for Pharmaceutical Technology at **Goethe University Frankfurt**.

In 2009, Kreuter's group reported that i.v. infusion of human serum albumin nanoparticles loaded with loperamide and covalently coupled to transferrin or transferrin receptor–binding antibodies induced anti-nociceptive effects in mice.[9] Loperamide is a generic opioid that does not cross the BBB under normal circumstances.

Both Gaillard and Kreuter cautioned against generalizing these results with the gold nanoparticle system to other nanoparticles.

Kreuter said that his own group's work has shown that conjugating the same targeting ligand to different types of nanoparticles can yield molecules that behave very differently from one another. He added that the agent to be delivered by the nanoparticle could itself significantly alter the nanoparticle's properties.

to-BBB uses liposomes coated with glutathione-conjugated polyethylene glycol (PEG) to improve drug delivery across the BBB. The company's 2B3-101, a liposomal formulation of doxorubicin coated with glutathione-conjugated PEG, is in Phase I/IIa testing to treat patients who have malignant glioma or solid tumors with metastasis to the brain.

The company plans to start the study's Phase IIa portion this year.

Genentech declined to comment.

Moving toward clinical relevance

Kreuter thinks an important next step will be identifying a relevant drug-

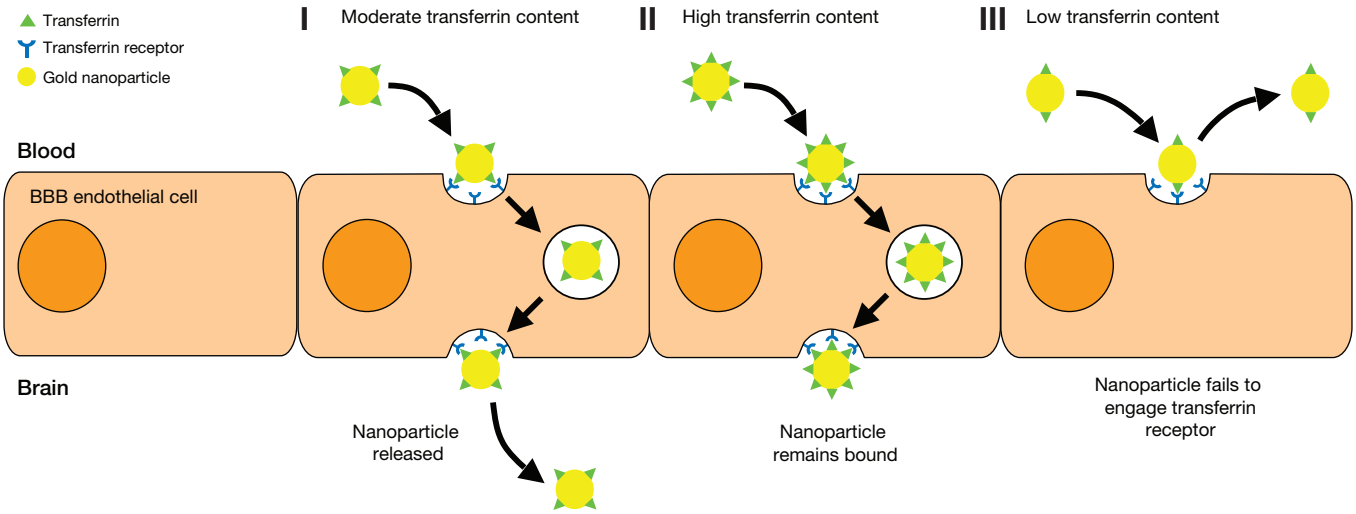

Figure 1. Model of receptor-mediated transcytosis of transferrin-containing nanoparticles. Transferrin and its receptor are one of the ligand-receptor combinations that could be exploited to transport molecules across the blood brain barrier (BBB). As reported in Wiley *et al.*, researchers found that gold nanoparticles with moderate transferrin content were most effective at crossing the BBB into the brain parenchyma. The researchers proposed a mechanism underlying their observations. Nanoparticles with moderate transferrin content (**I**) engage the transferrin receptor on BBB endothelial cells, undergo receptor-mediated transcytosis to traverse the BBB and are then released into the brain parenchyma. Nanoparticles with high transferrin content (**II**) remain associated with BBB endothelial cells and fail to be released into the brain parenchyma after receptor-mediated transcytosis. On the other hand, nanoparticles with low transferrin content (**III**) fail to engage the transferrin receptor on BBB endothelial cells.

nanoparticle combination and disease in which to test the transferrin-based approach.

Gaillard said that the researchers will need to do additional pharmacological legwork to confirm that their transferrin-coated nanoparticles are indeed crossing the BBB via receptor-mediated transcytosis and that the reported differences in reaching the brain parenchyma are due to variations in the nanoparticles' ability to bind the transferrin receptor.

"The researchers still need to show that the nanoparticles are not getting into the brain via some other mechanism or are the result of an artifact of the analytical method or experimental setup," he told *SciBX*.

Gaillard said that the Caltech researchers also must assess how the plasma and brain concentrations of transferrin-coated nanoparticles vary across multiple time points using pharmacologically relevant dosing regimens or at steady-state plasma levels. He also thinks that future studies should use nanoparticles that are more amenable than gold particles for drug delivery to the brain.

These would include nanoparticles created from biodegradable materials, such as poly(alkyl cyanoacrylates), poly(lactic-*co*-glycolic acid), albumin or chitosan, according to Kreuter.

Wiley said that the Caltech group is adapting the transferrin-based approach to polymeric nanoparticle systems that deliver therapeutic agents. The researchers are also trying to identify additional parameters that would be relevant to a nanoparticle's ability to cross the BBB and to understand how to modulate such parameters.

Wiley said that the potential therapeutic settings being considered are diseases of the brain where the BBB is known to exclude existing therapeutic agents, such as Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD) and brain cancer.

Caltech has filed for a patent covering methods to deliver nanoparticles to the brain. The technology is not available for licensing.

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REFERENCES

1. Wiley, D.T. *et al. Proc. Natl. Acad. Sci. USA*; published online May 6, 2013; doi:10.1073/pnas.1307152110
   Contact: Mark E. Davis, California Institute of Technology, Pasadena, Calif.
   e-mail: mdavis@cheme.caltech.edu
2. Descamps, L. *et al. Am. J. Physiol.* **270**, H1149–H1158 (1996)
3. Bickel, U. *et al. Adv. Drug Deliv. Rev.* **46**, 247–279 (2001)
4. Chang, J. *et al. Int. J. Pharm.* **379**, 285–292 (2009)
5. Karatas, H. *et al. J. Neurosci.* **29**, 13761–13769 (2009)
6. Osherovich, L. *SciBX* **4**(22); doi:10.1038/scibx.2011.619
7. Atwal, J.K. *et al. Sci. Transl. Med.* **3**, 84ra43 (2011)
8. Yu, Y.J. *et al. Sci. Transl. Med.* **3**, 84ra44 (2011)
9. Ulbrich, K. *et al. Eur. J. Pharm. Biopharm.* **71**, 251–256 (2009)

COMPANIES AND INSTITUTIONS MENTIONED

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