Arch. Pharm. Res. (2014) 37:24–30
DOI 10.1007/s12272-013-0272-6

Archives of Pharmacal Research
www.springer.com/12272

REVIEW

Nano-enabled delivery systems across the blood–brain barrier

Seung Rim Hwang · Kwangmeyung Kim

Received: 10 September 2013 / Accepted: 21 October 2013 / Published online: 30 October 2013

Abstract The development of drugs to treat disorders of the central nervous system (CNS) faces difficulties in achieving penetration of a drug through the blood–brain barrier (BBB) and allowing the drug to reach its intended target in the brain. There have been strategies to improve drug delivery to the brain through endogenous transport pathways such as passive diffusion, endocytosis, and active transport. Among various strategies, nano-enabled delivery systems offer a promising solution to improve the uptake and targeted delivery of drugs into the brain. Various nanocarriers including liposomes, bolaamphiphiles and nanoparticles can be used as a means to encapsulate drugs, either alone or in combination with targeting ligands. Moreover, most of materials used in nanocarrier fabrication are both biodegradable and biocompatible, thereby increasing the clinical utility of them. Here, we review the possibility to employ nano-enabled materials for delivery of drug across the BBB and the recent advances in nanotechnologies for therapy of the CNS diseases.

Keywords Central nervous system · Blood–brain barrier · Nanotechnology · Nano-enabled delivery · Receptor-mediated transcytosis

S. R. Hwang
College of Pharmacy, Chosun University, 309 Pilmun-daero, Dong-gu, Gwangju 501-759, South Korea

K. Kim (✉)
Center for Theragnosis, Biomedical Research Institute, Korea Institute of Science and Technology (KIST), Hwarangno 14-gil 6, Seongbuk-gu, Seoul 136-791, South Korea
e-mail: kim@kist.re.kr

Introduction

The number of research on drugs to treat disorders of the central nervous system (CNS) has sharply grown in the last decade; however, it takes long time to develop drugs and enter the market for the treatment of CNS disorders, such as depression, schizophrenia, insomnia, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, human immunodeficiency virus (HIV) infection of the brain, and brain tumors (Pardridge 2002; Palmer and Stephenson 2005). It may be largely attributed to a lack of appropriate drug delivery systems across the blood brain barrier (BBB) to ensure sufficient efficacy at the target site (Patel et al. 2009).

The BBB, composed of the cerebral endothelial cells, creates a unique extracellular fluid environment within the CNS, and is essential for the homeostasis of the brain (Begley 2004). The endothelial cells forming the BBB are characterized by tight continuous junctions, thus restricting penetration of polar solutes along the paracellular aqueous pathway through the junctions (Wolburg and Lippoldt 2002). Meanwhile, a transcellular pathway through the lipid membranes of the endothelium is taken for the free diffusion of O_2 and CO_2, which can be also a route of entry for small lipophilic agents such as barbiturates and ethanol. The endothelium also contains specific transport systems for glucose, amino acids, purine bases, nucleosides, choline and other small hydrophilic substances on the luminal and abluminal membranes, which provides a selective 'transport barrier' facilitating the entry of required nutrients and excluding potentially harmful compounds (Begley and Brightman 2003). Finally, intracellular and extracellular enzymes in cerebrovascular endothelial cells provide a 'metabolic barrier' against potentially penetrating lipophilic substances by metabolizing potentially toxic lipophilic substrates (El-Bacha and Minn 1999b).

Thus, the routes for transport across the BBB such as the paracellular aqueous pathway, transcellular lipophilic pathway, substrate-specific transport proteins, receptor-mediated transcytosis, and adsorptive-mediated transcytosis represent an invincible obstacle for most small-molecule drugs, peptides, and proteins to penetrate the brain (Abbott et al. 2006; Pardridge 2007). In an attempt to overcome these limitations, nanomaterials may be applied to deliver therapeutic agents across BBB (Kreuter 2001; Lockman et al. 2002; Silva 2008; Barbu et al. 2009). A wide variety of nanocarriers including dendrimers, micelles, liposomes, and polymeric nanoparticles has been proposed as drug delivery vehicles to improve delivery efficiency or drug kinetics and to reduce off-target effects.

In 1995, for the first time, intravenous injection of the polysorbate 80-coated nanoparticles was reported as being used for the delivery of the hexapeptide dalargin across the BBB (Alyautdin et al. 1995; Kreuter et al. 1995). Other drugs including loperamide, doxorubicin, and MRZ 2/576 (a *N*-methyl-$_D$-aspartate receptor antagonist) were also transported across the murine BBB by using poly(butylcyanoacrylate) nanoparticles (Alyautdin et al. 1997; Gulyaev et al. 1999; Friese et al. 2000).

Recently, researchers have been trying to build nano-enabled delivery of antineoplastic drugs to brain tumors in the CNS. For example, radiolabeled poly(ethylene glycol) (PEG)-coated hexadecylcyanoacrylate nanospheres were evaluated for accumulation in a rat gliosarcoma (Brigger et al. 2002). Preclinical data in brain cancer models also indicate that PEGylated liposomal doxorubicin with an additional glutathione (GSH) coating can significantly reduce brain tumor growth (Gaillard 2011). It should be noted that vascular endothelial cells are often abnormal and the BBB is defective in malignant gliomas (Schneider et al. 2004). Moreover, the failure in clearance of the excess fluid across the leaky BBB leads to brain edema, which contributes to patient morbidity (Papadopoulos et al. 2001).

This review summarizes the anatomical challenges of delivering drugs to the brain and the strategies using nanomaterials to enhance drug transportation into the CNS.

The blood–brain barrier (BBB)

The BBB, a separation of systemic blood circulation from the brain extracellular fluid, maintains the homeostasis of the CNS, and it is largely formed by endothelial cells of brain blood capillaries (Joo 1987). These cerebral endothelial cells have intercellular tight junction structures, which hinder the diffusion of hydrophilic macromolecules (DeVries et al. 1997). Importantly, the endothelial cells contain extracellular and intracellular enzymes such as peptidases, nucleotidases, monoamine oxidase, and several cytochrome P450 enzymes that afford a metabolic protection to the brain (el-Bacha and Minn 1999a). The BBB also includes astrocytic perivascular endfeet and a basal lamina embedded with pericytes that mediate the growth of endothelial cells, structural integrity, and cerebral autoregulation (Ballabh et al. 2004).

The existence of the BBB was first demonstrated when systemically injected chemical dyes by Ehrlich stained all of the organs of rats except for their brains. In contrast, in a later experiment by Goldmann, the brain tissue was stained after direct injection of dyes into the cerebro-spinal fluids of brains, indicating the existence of some kind of compartmentalization between the brain and the rest of the body. In 1955, it was reported that the hematoencephalic barrier was formed by the glial sheath covering the brain capillaries (Dempsey and Wislocki 1955). Administration of horseradish peroxidase (HRP) as an enzymatic tracer, however, revealed that the anatomical localization of the BBB could be detected at the level of the cerebral endothelial cells (Reese and Karnovsky 1967). This "barrier" results from tight junctions between endothelial cells in CNS vessels that exhibit various functional and morphological differences in comparison with peripheral organs. The tight junctions are composed of transmembrane proteins such as occludin, claudins, and junctional adhesion molecule, which are anchored by another protein complex (Butt et al. 1990).

There are several transport routes by which drugs may move across the BBB, including transcellular lipophilic diffusion, paracellular aqueous diffusion, carrier-mediated transport, receptor-mediated transcytosis, and adsorptive mediated transcytosis (Abbott et al. 2006; Abbott and Romero 1996). The BBB contains transport proteins as carriers for glucose, amino acids, purine bases, nucleosides, and choline. Among energy-dependent transporters, P-glycoprotein (Pgp) is a member of the ATP Binding Cassette (ABC) Superfamily, and displays poly-specificity for recognizing hydrophobic substrates as small as 330 up to 4,000 Da (Aller et al. 2009). The presence of Pgp associated with multidrug resistance in the BBB contributes the pharmacologic sanctuary for the entry of systemic drugs into the CNS. For example, the MDR1 Pgp expressed in the cerebral endothelium pumps out several drugs such as anthracyclines, Vinca alkaloids, etoposide and paclitaxel (Mayer et al. 1996). Drug transport by Pgp can be inhibited by reversal agents such as the cyclosporin A analogue, verapamil, quinidine, amiodarone, and SDZ PSC 833 (Ford 1996).

Meanwhile, nutrients such as iron, insulin, and leptin are taken up by specific receptor-mediated transport mechanisms (Jefferies et al. 1984; Duffy and Pardridge 1987; Golden et al. 1997). Receptor-mediated endocytosis and transcytosis involve the vesicular transfer of substances across cerebral endothelium, which requires binding of a

circulating ligand to a specific membrane receptor, followed by internalization (Jones and Shusta 2007). Adsorptive-mediated transcytosis is utilized in binding and uptake of cationic molecules such as peptides and proteins with a basic isoelectric point to the luminal surface of the BBB (Gabathuler 2010). The C-terminal structure and basicity of the molecules are more important than the number of constituent amino acids of peptides for the uptake by the adsorptive-mediated endocytosis (Tamai et al. 1997).

Recently, biopharmaceuticals including recombinant proteins, monoclonal antibodies, DNA, RNA or antisense oligonucleotides have gained an increasing interest as potential CNS agents; however, they would need effective brain targeting strategies because they cannot cross the BBB and reach the brain in effective amounts (Pardridge 2003; Pardridge 2010). These strategies will be discussed more in detail in the following section.

Nanotechnology for drug delivery to the brain

Approaches for delivery of drugs across the BBB may be broadly divided into the following categories: direct injection and implantation, chemical modifications, temporary opening of the BBB using permeability enhancers, nano-enabled delivery platforms via the intravenous route such as nanovesicles and nanoparticles coated with water-soluble surfactants or target ligands for specific receptor transporters, and intranasal delivery. Nanotechnology used for each approach will be described in each category.

Direct injection and implantation

To bypass the BBB, CNS drugs can be delivered to the target tissue by direct intrathecal injection into the cerebrospinal fluid (CSF) or by interstitial delivery through intracerebral implantation. Intrathecal delivery widely used for anesthesia and acute cancer pain management minimizes the risk of systemic side-effects. However, the rapid clearance of CSF restricts the diffusion of drugs into the CNS tissue (Pathan et al. 2009). Polymeric nanocomposite hydrogels for sustained local delivery have been developed to overcome these limitations (Baumann et al. 2010). The nanoscale intracerebral implants also demonstrate the potential for enhancing drug pharmacokinetics and therapeutic efficacy (Ranganath et al. 2010).

Chemical modifications

Physicochemical properties involved in passive diffusion of CNS drugs across the BBB are the lipophilicity, polar surface area and molecular weight; any drugs that do not meet certain criteria for passive diffusion may require more transport system to reach the brain (Mensch et al. 2009). Several chemical modifications such as lipidation, cationization, and prodrug approach have been incorporated into drugs to improve the penetration into brain. In addition, nanoparticulate vectors of drugs also can be chemically modified to increase cellular uptake and the potential delivery in different cell compartments (Juillerat-Jeanneret 2008). Anti-cancer agents have been loaded in nanocontainers conjugated with ligands targeting the BBB to enhance selectivity for brain cancers (Soni et al. 2005).

Permeability enhancers

Gap junction proteins could be potential targets for modulating the paracellular flux of mannitol and insulin and the barrier function of brain endothelial cells (Deli 2009). Vasoactive compounds, such as histamine, bradykinin, or leukotrienes increase BBB permeability, especially in blood vessels in brain tumour tissue. Alkermes Incorporation has developed a synthetic bradykinin analog, Cereport (RMP-7), that increases cyclic GMP levels through binding to the cerebrovascular bradykinin B_2 receptor, transiently disrupts the tight junctions in brain endothelial cells, and increases brain permeability (Bartus et al. 2000; Emerich et al. 2000). Methylmethacrylate-sulfopropylmethacrylate nanoparticles with grafted RMP-7 to deliver antiviral agents such as stavudine, delavirdine and saquinavir demonstrated an increased uptake in a co-culture model containing human brain-microvascular endothelial cells and astrocytes (Kuo and Lee 2012). It is mainly due to endocytosis of nanoparticles grafted with RMP-7 by endothelial cells, which improves the transport of drugs into the brain.

Nano-enabled delivery platforms via the intravenous route

Nano-enabled delivery systems that utilize lipid-based carriers at sizes between 1 and 100 nm have been designed to improve drug delivery to the brain. Several groups have shown that doxorubicin as liposomal encapsulated formulation (Caelyx®/Doxil®) is effective in treating patients with malignant glioma (Fabel et al. 2001; Hau et al. 2004; Ranganathan et al. 2012). The presence of endogenous receptors in the brain can be targeted by nanosized drug loaded liposomes. For example, GSH, an antioxidant tripeptide preventing damage to important cellular components, has specific binding sites throughout the brain and spinal cord (Guo et al. 1992; Lanius et al. 1994; Kannan et al. 2000). Thus, GSH can be linked to drug-loaded carriers such as liposomes and nanoparticles for targeting

to the brain (Vlieghe and Khrestchatisky 2010). 2B3-101 (GSH PEGylated liposomal doxorubicin) and 2B3-201 (GSH PEGylated liposomal methylprednisolone) showed therapeutic benefits and predictable safety profiles.

It has been demonstrated that the cationic nanosized vesicles made from bolaamphiphiles can penetrate the BBB and allow targeted delivery and controlled release of drugs. The bolaamphiphiles with hydrophilic groups at both ends of a hydrophobic chain form monolayer membrane vesicles and show high encapsulation capacity and stability, while classical bilayer membrane liposomes made of phospholipid cannot efficiently entrap drugs (Popov et al. 2010; Popov et al. 2012; Dakwar et al. 2012).

Like liposomes, solid colloidal matrix-like nanoparticles made of either polymers or lipids are also generally administered via the intravenous route (Soppimath et al. 2001; Wissing et al. 2004). They are relatively simple to manufacture, exhibit high physical stability, and can be used for the prolonged drug release. The properties of nanoparticles for brain drug delivery are related to the following manufacturing factors: biodegradability and biocompatibility of polymers and surfactants, nanoparticle diameter, physical stability in blood, and drug molecules. Physiological factors such as the mononuclear phagocyte system and receptor-mediated transcytosis also influence the amount of drug delivered into the brain (Lockman et al. 2002). A good in vitro-sustained release was achieved with nanoparticles made of methoxypoly(ethylene glycol)-polylactide or poly(lactide-co-glycolide) (mPEG-PLA/PLGA) (Olivier 2005).

Despite effectiveness in delivering drug to the brain, nanoparticles have potential toxicity that may limit their clinical applications. Brain-targeted PEGylated immunonanoparticles can be synthesized using targeting ligands such as peptidomimetic monoclonal antibodies to BBB transcytosis receptor that is highly expressed on the brain capillary endothelium (Huwyler et al. 1996). In addition, there is ongoing controversy concerning the exact transport mechanism of nanoparticles. It was hypothesized that polysorbate-coated nanoparticles would stick to serum lipoproteins such as apolipoprotein E, leading to endocytosis by the brain endothelial cells (Allemann et al. 1997).

More recently, PEGylated carbon nanotubes, nanoparticles, and liposomes modified with angiopep-2 targeting low-density lipoprotein receptor-related protein receptor have been used to improve target ability (Ren et al. 2012; Xin et al. 2012; Sun et al. 2012). Furthermore, the pharmaceutical companies such as Angiochem and Geron have successfully applied nano-enabled technologies in delivering drugs for the treatment of CNS diseases. The existing strategies are mainly harnessing transport routes such as receptor-mediated transcytosis to improve the uptake and targeted delivery into the brain. Several liposomal formulations of approved chemotherapeutics are under development, and they could make a significant impact on the treatment paradigm of CNS diseases.

Intranasal delivery

Direct intranasal delivery of therapeutics is a non-invasive route that provides a rapid onset of action, direct nose-to-brain delivery along the olfactory and trigeminal nerves, and minimization of systemic exposure and metabolism. Intranasal administration of encapsulated small-molecule drugs, peptides and proteins within nanoparticles has also shown enhanced drug delivery into the brain. Zhang et al. (2006) showed that the intranasally administered MPEG-PLA nanoparticles resulted in the enhanced uptake of drugs into the CSF. It also has been reported that PEG-PLGA nanoparticles coated with odorranalectin, a small peptide lectin with low immunogenicity, could be potentially used as a nose-to-brain drug delivery carrier, as could lactoferrin conjugated PEG-PLGA nanoparticles and poly(ethyleneglycol)-poly(epsilon-caprolactone) polymersomes conjugated with mouse-anti-rat monoclonal antibody OX26 (Wen et al. 2011; Hu et al. 2011; Pang et al. 2008).

Conclusion

Nano-enabled delivery systems have been proved to efficiently deliver therapeutics across the BBB. They can be designed to shield the encapsulated active compounds from systemic side effects by crossing the BBB and target specific cells. Currently, several nanotechnology-based approaches for the treatment of CNS diseases are already in clinical trials, and therefore have great potential for making impact on the clinic application. However, more detailed research is necessary to determine the safety profiles and exact transport mechanisms of them.

The most accepted mechanism for the brain uptake of nanoparticles now appears to be transcytosis mediated by specific receptors expressed at the BBB (Gabathuler 2010). Lipoprotein as well as the transferrin receptor and insulin receptor can be utilized for targeted drug delivery. It should be also noted that basement membrane and associated pericytes are often abnormal in tumors and that the BBB is defective in malignant gliomas. Furthermore, nano-enabled delivery systems open up new possibilities for biopharmaceuticals that would need effective brain targeting strategies as potential CNS agents.

Acknowledgments This work was supported by the Intramural Research Program (Theragnosis, Global RNAi Carrier Initiative, and KIST Young Fellow) of KIST.

References

Abbott, N.J., and I.A. Romero. 1996. Transporting therapeutics across the blood–brain barrier. *Molecular Medicine Today* 2: 106–113.

Abbott, N.J., L. Ronnback, and E. Hansson. 2006. Astrocyte-endothelial interactions at the blood–brain barrier. *Nature Reviews Neuroscience* 7: 41–53.

Allemann, E., P. Gravel, J.C. Leroux, L. Balant, and R. Gurny. 1997. Kinetics of blood component adsorption on poly(D, L-lactic acid) nanoparticles: Evidence of complement C3 component involvement. *Journal of Biomedical Materials Research* 37: 229–234.

Aller, S.G., J. Yu, A. Ward, Y. Weng, S. Chittaboina, R. Zhuo, P.M. Harrell, Y.T. Trinh, Q. Zhang, I.L. Urbatsch, and G. Chang. 2009. Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. *Science* 323: 1718–1722.

Alyautdin, R., D. Gothier, V. Petrov, D. Kharkevich, and J. Kreuter. 1995. Analgesic activity of the hexapeptide dalargin adsorbed on the surface of polysorbate 80-coated poly(butyl cyanoacrylate) nanoparticles. *European Journal of Pharmaceutics and Biopharmaceutics* 41: 44–48.

Alyautdin, R.N., V.E. Petrov, K. Langer, A. Berthold, D.A. Kharkevich, and J. Kreuter. 1997. Delivery of loperamide across the blood–brain barrier with polysorbate 80-coated polybutylcyanoacrylate nanoparticles. *Pharmaceutical Research* 14: 325–328.

Ballabh, P., A. Braun, and M. Nedergaard. 2004. The blood–brain barrier: An overview: Structure, regulation, and clinical implications. *Neurobiology of Disease* 16: 1–13.

Barbu, E., E. Molnar, J. Tsibouklis, and D.C. Gorecki. 2009. The potential for nanoparticle-based drug delivery to the brain: Overcoming the blood–brain barrier. *Expert Opinion on Drug Delivery* 6: 553–565.

Bartus, R.T., P. Snodgrass, J. Marsh, M. Agostino, A. Perkins, and D.F. Emerich. 2000. Intravenous cereport (RMP-7) modifies topographic uptake profile of carboplatin within rat glioma and brain surrounding tumor, elevates platinum levels, and enhances survival. *The Journal of Pharmacology and Experimental Therapeutics* 293: 903–911.

Baumann, M.D., C.E. Kang, C.H. Tator, and M.S. Shoichet. 2010. Intrathecal delivery of a polymeric nanocomposite hydrogel after spinal cord injury. *Biomaterials* 31: 7631–7639.

Begley, D.J. 2004. Delivery of therapeutic agents to the central nervous system: The problems and the possibilities. *Pharmacology Therapeut* 104: 29–45.

Begley, D.J., and M.W. Brightman. 2003. Structural and functional aspects of the blood–brain barrier. Progress in drug research. Fortschritte der Arzneimittelforschung. *Progres des Recherches Pharmaceutiques* 61: 39–78.

Brigger, I., J. Morizet, G. Aubert, H. Chacun, M.J. Terrier-Lacombe, P. Couvreur, and G. Vassal. 2002. Poly(ethylene glycol)-coated hexadecylcyanoacrylate nanospheres display a combined effect for brain tumor targeting. *Journal of Pharmacology and Experimental Therapeutics* 303: 928–936.

Butt, A.M., H.C. Jones, and N.J. Abbott. 1990. Electrical resistance across the blood–brain barrier in anaesthetized rats: A developmental study. *The Journal of Physiology* 429: 47–62.

Dakwar, G.R., I. Abu Hammad, M. Popov, C. Linder, S. Grinberg, E. Heldman, and D. Stepensky. 2012. Delivery of proteins to the brain by bolaamphiphilic nano-sized vesicles. *The Journal of Controlled Release* 160: 315–321.

Deli, M.A. 2009. Potential use of tight junction modulators to reversibly open membranous barriers and improve drug delivery. *Biochimica et Biophysica Acta* 1788: 892–910.

Dempsey, E.W., and G.B. Wislocki. 1955. An electron microscopic study of the blood–brain barrier in the rat, employing silver nitrate as a vital stain. *The Journal of Biophysical and Biochemical Cytology* 1: 245–256.

Devries, H.E., J. Kuiper, A.G. Deboer, T.J.C. Vanberkel, and D.D. Breimer. 1997. The blood–brain barrier in neuroinflammatory diseases. *Pharmacological Reviews* 49: 143–155.

Duffy, K.R., and W.M. Pardridge. 1987. Blood–brain barrier transcytosis of insulin in developing rabbits. *Brain Research* 420: 32–38.

El-Bacha, R.S., and A. Minn. 1999a. Drug metabolizing enzymes in cerebrovascular endothelial cells afford a metabolic protection to the brain. *Cellular and Molecular Biology (Noisy-le-grand)* 45: 15–23.

El-Bacha, R.S., and A. Minn. 1999b. Drug metabolizing enzymes in cerebrovascular endothelial cells afford a metabolic protection to the brain. *Cellular and Molecular Biology* 45: 15–23.

Emerich, D.F., R.L. Dean, J. Marsh, M. Pink, D. Lafreniere, P. Snodgrass, and R.T. Bartus. 2000. Intravenous cereport (RMP-7) enhances delivery of hydrophilic chemotherapeutics and increases survival in rats with metastatic tumors in the brain. *Pharmaceutical Research* 17: 1212–1219.

Fabel, K., J. Dietrich, P. Hau, C. Wismeth, B. Winner, S. Przywara, A. Steinbrecher, W. Ullrich, and U. Bogdahn. 2001. Long-term stabilization in patients with malignant glioma after treatment with liposomal doxorubicin. *Cancer* 92: 1936–1942.

Ford, J.M. 1996. Experimental reversal of P-glycoprotein-mediated multidrug resistance by pharmacological chemosensitisers. *European Journal of Cancer* 32A: 991–1001.

Friese, A., E. Seiller, G. Quack, B. Lorenz, and J. Kreuter. 2000. Increase of the duration of the anticonvulsive activity of a novel NMDA receptor antagonist using poly(butylcyanoacrylate) nanoparticles as a parenteral controlled release system. *European Journal of Pharmaceutics and Biopharmaceutics* 49: 103–109.

Gabathuler, R. 2010. Approaches to transport therapeutic drugs across the blood–brain barrier to treat brain diseases. *Neurobiology of Disease* 37: 48–57.

Gaillard, P.J. 2011. Case study: To-BBB's G-technology, getting the best from drug-delivery research with industry-academia partnerships. *Therapeutic Delivery* 2: 1391–1394.

Golden, P.L., T.J. Maccagnan, and W.M. Pardridge. 1997. Human blood–brain barrier leptin receptor. Binding and endocytosis in isolated human brain microvessels. *The Journal of Clinical Investigation* 99: 14–18.

Gulyaev, A.E., S.E. Gelperina, I.N. Skidan, A.S. Antropov, G.Y. Kivman, and J. Kreuter. 1999. Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles. *Pharmaceutical Research* 16: 1564–1569.

Guo, N., C. Mcintosh, and C. Shaw. 1992. Glutathione: New candidate neuropeptide in the central nervous system. *Neuroscience* 51: 835–842.

Hau, P., K. Fabel, U. Baumgart, P. Rummele, O. Grauer, A. Bock, C. Dietmaier, W. Dietmaier, J. Dietrich, C. Dudel, F. Hubner, T. Jauch, E. Drechsel, I. Kleiter, C. Wismeth, A. Zellner, A. Brawanski, A. Steinbrecher, J. Marienhagen, and U. Bogdahn. 2004. Pegylated liposomal doxorubicin-efficacy in patients with recurrent high-grade glioma. *Cancer* 100: 1199–1207.

Hu, K., Y. Shi, W. Jiang, J. Han, S. Huang, and X. Jiang. 2011. Lactoferrin conjugated PEG-PLGA nanoparticles for brain delivery: Preparation, characterization and efficacy in Parkinson's disease. *International Journal of Pharmaceutics* 415: 273–283.

Huwyler, J., D. Wu, and W.M. Pardridge. 1996. Brain drug delivery of small molecules using immunoliposomes. *Proceedings of the National Academy of Sciences USA* 93: 14164–14169.

Jefferies, W.A., M.R. Brandon, S.V. Hunt, A.F. Williams, K.C. Gatter, and D.Y. Mason. 1984. Transferrin receptor on endothelium of brain capillaries. *Nature* 312: 162–163.

Jones, A.R., and E.V. Shusta. 2007. Blood–brain barrier transport of therapeutics via receptor-mediation. *Pharmaceutical Research* 24: 1759–1771.

Joo, F. 1987. The blood–brain-barrier. *Nature* 329: 208.

Juillerat-Jeanneret, L. 2008. The targeted delivery of cancer drugs across the blood–brain barrier: Chemical modifications of drugs or drug-nanoparticles? *Drug Discovery Today* 13: 1099–1106.

Kannan, R., R. Chakrabarti, D. Tang, K.J. Kim, and N. Kaplowitz. 2000. GSH transport in human cerebrovascular endothelial cells and human astrocytes: Evidence for luminal localization of Na+-dependent GSH transport in HCEC. *Brain Research* 852: 374–382.

Kreuter, J. 2001. Nanoparticulate systems for brain delivery of drugs. *Advanced Drug Delivery Reviews* 47: 65–81.

Kreuter, J., R.N. Alyautdin, D.A. Kharkevich, and A.A. Ivanov. 1995. Passage of peptides through the blood–brain-barrier with colloidal polymer particles (nanoparticles). *Brain Research* 674: 171–174.

Kuo, Y.C., and C.L. Lee. 2012. Methylmethacrylate-sulfopropyl-methacrylate nanoparticles with surface RMP-7 for targeting delivery of antiretroviral drugs across the blood–brain barrier. *Colloid Surface B* 90: 75–82.

Lanius, R.A., C.A. Shaw, R. Wagey, and C. Krieger. 1994. Characterization, distribution, and protein kinase C-mediated regulation of [35S]glutathione binding sites in mouse and human spinal cord. *Journal of Neurochemistry* 63: 155–160.

Lockman, P.R., R.J. Mumper, M.A. Khan, and D.D. Allen. 2002. Nanoparticle technology for drug delivery across the blood–brain barrier. *Drug Development and Industrial Pharmacy* 28: 1–13.

Mayer, U., E. Wagenaar, J.H. Beijnen, J.W. Smit, D.K.F. Meijer, J. Vanasperen, P. Borst, and A.H. Schinkel. 1996. Substantial excretion of digoxin via the intestinal mucosa and prevention of long-term digoxin accumulation in the brain by the mdr1a P-glycoprotein. *British Journal of Pharmacology* 119: 1038–1044.

Mensch, J., J. Oyarzabal, C. Mackie, and P. Augustijns. 2009. In vivo, in vitro and in silico methods for small molecule transfer across the BBB. *Journal of Pharmaceutical Sciences* 98: 4429–4468.

Olivier, J.C. 2005. Drug transport to brain with targeted nanoparticles. *The Journal of the American Society for Experimental Neuro Therapeutics* 2: 108–119.

Palmer, A.M., and F.A. Stephenson. 2005. CNS drug discovery: Challenges and solutions. *Drug News and Perspectives* 18: 51–57.

Pang, Z., W. Lu, H. Gao, K. Hu, J. Chen, C. Zhang, X. Gao, X. Jiang, and C. Zhu. 2008. Preparation and brain delivery property of biodegradable polymersomes conjugated with OX26. *The Journal of Controlled Release* 128: 120–127.

Papadopoulos, M.C., S. Saadoun, D.C. Davies, and B.A. Bell. 2001. Emerging molecular mechanisms of brain tumour oedema. *British Journal of Neurosurgery* 15: 101–108.

Pardridge, W.M. 2002. Why is the global CNS pharmaceutical market so under-penetrated? *Drug Discovery Today* 7: 5–7.

Pardridge, W.M. 2003. Blood–brain barrier drug targeting: The future of brain drug development. *Molecular Interventions* 3: 90–105.

Pardridge, W.M. 2007. Drug targeting to the brain. *Pharmaceutical Research* 24: 1733–1744.

Pardridge, W.M. 2010. Biopharmaceutical drug targeting to the brain. *Journal of Drug Targeting* 18: 157–167.

Patel, M.M., B.R. Goyal, S.V. Bhadada, J.S. Bhatt, and A.F. Amin. 2009. Getting into the brain approaches to enhance brain drug delivery. *Central Nervous System Drugs* 23: 35–58.

Pathan, S.A., Z. Iqbal, S.M. Zaidi, S. Talegaonkar, D. Vohra, G.K. Jain, A. Azeem, N. Jain, J.R. Lalani, R.K. Khar, and F.J. Ahmad. 2009. CNS drug delivery systems: Novel approaches. *Recent Patents on Drug Delivery and Formulation* 3: 71–89.

Popov, M., S. Grinberg, C. Linder, T. Waner, B. Levi-Hevroni, R.J. Deckelbaum, and E. Heldman. 2012. Site-directed decapsulation of bolaamphiphilic vesicles with enzymatic cleavable surface groups. *The Journal of Controlled Release* 160: 306–314.

Popov, M., C. Linder, R.J. Deckelbaum, S. Grinberg, I.H. Hansen, E. Shaubi, T. Waner, and E. Heldman. 2010. Cationic vesicles from novel bolaamphiphilic compounds. *Journal of Liposome Research* 20: 147–159.

Ranganath, S.H., Y. Fu, D.Y. Arifin, I. Kee, L. Zheng, H.S. Lee, P.K. Chow, and C.H. Wang. 2010. The use of submicron/nanoscale PLGA implants to deliver paclitaxel with enhanced pharmacokinetics and therapeutic efficacy in intracranial glioblastoma in mice. *Biomaterials* 31: 5199–5207.

Ranganathan, R., S. Madanmohan, A. Kesavan, G. Baskar, Y.R. Krishnamoorthy, R. Santosham, D. Ponraju, S.K. Rayala, and G. Venkatraman. 2012. Nanomedicine: Towards development of patient-friendly drug-delivery systems for oncological applications. *International Journal of Nanomedicine* 7: 1043–1060.

Reese, T.S., and M.J. Karnovsky. 1967. Fine structural localization of a blood–brain barrier to exogenous peroxidase. *The Journal of Cell Biology* 34: 207–217.

Ren, J., S. Shen, D. Wang, Z. Xi, L. Guo, Z. Pang, Y. Qian, X. Sun, and X. Jiang. 2012. The targeted delivery of anticancer drugs to brain glioma by PEGylated oxidized multi-walled carbon nanotubes modified with angiopep-2. *Biomaterials* 33: 3324–3333.

Schneider, S.W., T. Ludwig, L. Tatenhorst, S. Braune, H. Oberleithner, V. Senner, and W. Paulus. 2004. Glioblastoma cells release factors that disrupt blood–brain barrier features. *Acta Neuropathologica* 107: 272–276.

Silva, G.A. 2008. Nanotechnology approaches to crossing the blood–brain barrier and drug delivery to the CNS. *BMC Neuroscience* 9(Suppl 3): S4. doi:10.1186/1471-2202-9-S3-S4.

Soni, V., D.V. Kohli, and S.K. Jain. 2005. Transferrin coupled liposomes as drug delivery carriers for brain targeting of 5-florouracil. *Journal of Drug Targeting* 13: 245–250.

Soppimath, K.S., T.M. Aminabhavi, A.R. Kulkarni, and W.E. Rudzinski. 2001. Biodegradable polymeric nanoparticles as drug delivery devices. *The Journal of Controlled Release* 70: 1–20.

Sun, X., Z. Pang, H. Ye, B. Qiu, L. Guo, J. Li, J. Ren, Y. Qian, Q. Zhang, J. Chen, and X. Jiang. 2012. Co-delivery of pEGFP-hTRAIL and paclitaxel to brain glioma mediated by an angiopep-conjugated liposome. *Biomaterials* 33: 916–924.

Tamai, I., Y. Sai, H. Kobayashi, M. Kamata, T. Wakamiya, and A. Tsuji. 1997. Structure-internalization relationship for adsorptive-mediated endocytosis of basic peptides at the blood–brain barrier. *The Journal of Pharmacology and Experimental Therapeutics* 280: 410–415.

Vlieghe, P., and M. Khrestchatisky. 2010. Peptide-based vectors for blood–brain barrier targeting and delivery of drugs to the central nervous system. *Therapeutic Delivery* 1: 489–494.

Wen, Z., Z. Yan, R. He, Z. Pang, L. Guo, Y. Qian, X. Jiang, and L. Fang. 2011. Brain targeting and toxicity study of odorranalectin-conjugated nanoparticles following intranasal administration. *Drug Delivery* 18: 555–561.

Wissing, S.A., O. Kayser, and R.H. Muller. 2004. Solid lipid nanoparticles for parenteral drug delivery. *Advanced Drug Delivery Reviews* 56: 1257–1272.

Wolburg, H., and A. Lippoldt. 2002. Tight junctions of the blood–brain barrier: Development, composition and regulation. *Vascular Pharmacology* 38: 323–337.

Xin, H., X. Sha, X. Jiang, L. Chen, K. Law, J. Gu, Y. Chen, X. Wang, and X. Fang. 2012. The brain targeting mechanism of Angiopep-conjugated poly(ethylene glycol)-co-poly(epsilon-caprolactone) nanoparticles. *Biomaterials* 33: 1673–1681.

Zhang, Q.Z., L.S. Zha, Y. Zhang, W.M. Jiang, W. Lu, Z.Q. Shi, X.G. Jiang, and S.K. Fu. 2006. The brain targeting efficiency following nasally applied MPEG-PLA nanoparticles in rats. *Journal of Drug Targeting* 14: 281–290.