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COMMENTARY

Cancer and the blood–brain barrier: 'Trojan horses' for courses?

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The blood–brain barrier (BBB) limits the bioavailability of most bioactive molecules and drugs in the CNS, leaving clinicians with only a few options for pharmacotherapy. In this issue Regina *et al.* demonstrate that a 'Trojan horse' drug conjugate, acting as a substrate of a physiological BBB receptor that facilitates transcytosis, significantly improves drug transport into the CNS. Specifically, the low-density lipoprotein receptor-related protein (LRP) is used to carry a conjugate of paclitaxel and Angiopep-2, an aprotinin-derived peptide, across the BBB. Interestingly, in its conjugated form paclitaxel circumvents the efflux pumps at the BBB but still retains its activity against microtubules. Importantly, the authors were able to demonstrate improved therapeutic efficacy of this approach in orthotopic models of primary and metastatic brain cancer. This proof-of-principle study thus represents a milestone for drug delivery across the BBB but also a starting point for studies exploring wider applicability and potential limitations of the approach.
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Abbreviations: APP, secreted β-amyloid protein; Aβ, amyloid-β; BBB, blood–brain barrier; KPI, Kunitz Protease Inhibitor; LRP, low-density lipoprotein receptor-related protein; P-gp, P-glycoprotein

Current CNS drug treatments predominantly address four CNS disorders: depression, schizophrenia, chronic pain and epilepsy, whereas treatment options for brain cancer, neurodegenerative diseases and most other CNS conditions are quite limited (Pardridge, 2005). These limitations can be directly linked to the blood–brain barrier (BBB), a physical, metabolic and immunological barrier formed by endothelial cells, pericytes, astrocytes and the basal membrane. It controls the transport of molecules from the blood circulation to the brain parenchyma so effectively that around 95% of more than 7000 small bioactive compounds (MW < 500) covered by the Comprehensive Medicinal Chemistry database (and 100% of large bioactive molecules) have insufficient bioavailability in the brain (Pardridge, 2005).

When considering potential approaches to increasing CNS drug bioavailability, it is important to link our increasingly detailed understanding of the mechanisms and molecules, which control the BBB with the clinical needs of the specific condition to be treated. Although for some conditions local delivery strategies may be suitable, for example, for the therapy of highly localized and acute diseases, others, such as many neurodegenerative diseases, may require chronic treatment of larger brain regions or the whole CNS.

In this context cancer represents a somewhat special challenge: its tendency for invasive growth and metastasis means that it typically evolves from a local to a systemic disease. Furthermore, curative treatment requires a therapy to be 100% effective, as even a few cells with stem cell-like properties would otherwise be able to regenerate the tumour.

Worldwide, 189 000 primary brain tumours and, in addition, about double that number of brain metastases (~170 000 new cases in the US alone) are newly diagnosed every year. Both, primary and metastatic brain tumours, have a poor prognosis and mean patient survival can be as low as 9 months after diagnosis.

Although the BBB also limits the CNS bioavailability of anticancer drugs, it is interesting to note that the clinical response rates of peripheral tumours and the corresponding CNS metastases are in fact often quite similar (Deeken and Loscher, 2007). This suggests that current drugs are, in principle, effective and, that therapeutic drug concentrations in the CNS are actually achievable. The explanation for this apparent paradox lies in the fact that a substantial breakdown of the BBB occurs, typically at the more advanced stage of the disease. However, because of the restricted transport within the brain parenchyma, treatment of tumour cells is likely to remain limited to the immediate vicinity of

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the leaky barrier. Consequently, more distant patches of locally advanced disease or metastases remain essentially untouched, thus rendering the tumour primed for a relapse.

This scenario means that the key challenges of successful brain cancer treatment can be directly linked to the lack of delivery strategies able to overcome the BBB in a global—rather than local—fashion.

Under physiological conditions, control of transport across the BBB is achieved as follows:

(a) minimization of transbarrier transport (for example, through continuous tight junctions, lack of fenestrations and minimal pinocytosis of brain endothelial cells);
(b) active expulsion of the unwanted molecules that cross the barrier by efflux pumps (for example, P-glycoprotein (P-gp), members of the multi resistance protein family, organic anion and cation transporters) and
(c) selective and active transport of the required substrates through a range of transporters and internalizing receptors.

Not surprisingly, delivery strategies to overcome the BBB utilize all of these mechanisms in an effort to increase CNS drug bioavailability (de Boer and Gaillard, 2007), by increasing lipid diffusion (Qu *et al*., 2006), temporary opening of the barrier pharmacologically or through osmotic shock, by coadministration of chemotherapeutic agents with efflux pump inhibitors, or coupling of drugs and drug carriers to the endogenous substrates of various transporters/receptors or the corresponding antibodies (e.g. OX26).

In the current issue of the *BJP,* Regina *et al*. (2008) demonstrate that the latter approach based on ANG1005, a 'Trojan horse' paclitaxel conjugate posing as substrate of a BBB receptor that facilitates transcytosis, significantly improves survival in orthotopic models of glioma and of brain metastases. ANG1005 is formed by conjugation of paclitaxel to the peptide Angiopep-2, a ligand of the low-density lipoprotein receptor-related protein (LRP).

The physiological role of LRP receptors appears to involve the control of the permeability of the BBB, vascular tone and post-ischemic lesion formation in response to an active tissue-type plasminogen activator (Herz, 2003). LRP1 is an ubiquitously expressed receptor which binds more than 40 different ligands ranging from lipoproteins, protease/protease inhibitor complexes, extracellular matrix proteins and viruses to growth factors and cytokines (May *et al*., 2005). LRP2, or megalin, is like LRP1 a scavenger and multi-ligand receptor, expressed by many resorptive epithelia, thus indicating a role in endocytosis and transport (May *et al*., 2005).

Demeule and colleagues have previously identified the peptide sequence responsible for binding and transport through LRP receptors and selected Angiopep-2 as a potential carrier for brain delivery (Demeule *et al*., 2008). Angiopep-2, is a member of an Aprotinin-derived peptide family that share a particular peptidic sequence, the Kunitz domain, with other protein ligands for LRP: secreted β-amyloid protein (APP), bikunin and Kunitz protease inhibitor. Regina *et al*. (2008) linked Angiopep-2 through an ester bond to the anticancer drug paclitaxel.

Although it was known that paclitaxel has activity against malignant glioma and brain metastases (Fellner *et al*., 2002) its clinical use has been hampered by the fact that it is a substrate of P-gp, a multidrug resistance efflux pump, which limits the penetration of drugs that have entered from the blood circulation deeper into the CNS. Initial attempts to increase drug concentrations in the parenchyma by coadministration of efflux pump inhibitors have been abandoned after unsuccessful Phase III trials and approaches that circumvent rather than inhibit these efflux pumps are therefore an interesting alternative.

Regina *et al*. (2008) show that the conjugation of paclitaxel to Angiopep-2 results in a 'Trojan horse' drug that exploits physiological mechanisms to increase CNS bioavailability; the brain uptake of the conjugate ANG1005 is significantly higher than that of paclitaxel and is not affected by the activity of the P-gp efflux pump. Interestingly, ANG1005's antiproliferative activity does not seem to require release of the conjugated paclitaxel; the conjugate retains the ability to stabilize the microtubules. The authors were able to demonstrate preclinical proof-of-principle in relevant orthotopic tumour models.

Taken together, these advances in terms of delivery to the brain hold considerable promise for the clinic. The study also raises a number of questions relevant to the clinical development of this approach and related strategies.

Current anticancer drugs tend to be cytotoxic compounds of limited specificity, a fact which is associated with a wide range of potential side effects and even dose-limiting toxicity. Changes in a drug's biodistribution and bioavailability, which are frequently brought about by delivery systems potentially also induce changes in the side effect profile, for instance, the occurrence of foot–hand syndrome with liposomal doxorubicin. In this context it will thus be important to understand the potential effects of changes in the biodistribution because of the conjugation and targeting.

In a wider context, this is also relevant to other strategies, which are using transporters/receptors that are active at the BBB but also have a physiological role in other tissues.

In the case of Angiopep-2 such sites/functions might include the liver, where LRP1 serves as a hepatocyte receptor involved in the lipid metabolism, vascular smooth muscle cells, macrophages and but also the coagulation-fibrinolysis system (Herz, 2003; de Boer and Gaillard, 2007). LRP2 on the other hand is also expressed on the apical plasma membrane of absorptive and secretory epithelia, as found in renal proximal tubules, thyroid and parathyroid glands, trophoectoderm and neuroectoderm (May *et al*., 2005). High levels of LRP-type receptors are also present on gliomas but it is currently unclear whether this may affect the pharmacology of ANG1005 (Yamamoto *et al*., 1998).

For the chronic treatment of CNS conditions using 'Trojan horse' strategies such as ANG1005 the potential involvement of the respective transporters/receptors in the pathology of various diseases and brain conditions may also need to be considered. For example, LRP has been implicated in Alzheimer's disease as a factor influencing the metabolism of amyloid-β (Aβ) but also as a receptor for APP, apoE and α2/microtubulin (Herz, 2003; de Boer and Gaillard, 2007). Furthermore, it has also been implicated in the progression of atherosclerotic lesions (Llorente-Cortes and Badimon, 2005).

The work of Regina *et al*. (2008) in the current issue represents an important milestone in terms of drug delivery across the BBB and the development of therapies for brain cancer. It demonstrates that Trojan horse strategies which exploit binding of drug conjugates to physiological transport receptors at the BBB are not only able to achieve increased drug transport across the BBB but also deliver improved therapeutic outcomes in relevant animal models. It is now important to explore some of the remaining questions in more detail to determine whether 'Trojan horse' systems such as ANG1005 will have a general role to play in CNS drug therapy or are more suited to specific 'courses', i.e. selected drug/disease combinations.

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