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Anticancer drug delivery to the brain: Outwitting the blood-brain barrier

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Abstract

Brain tumors are responsible for almost 2% of deaths in the Western world. The most used methods nowadays to treat a malignant brain tumor are surgery, radiotherapy and chemotherapy, generally applied in combination. The prognosis for patients suffering from a highly malignant glioblastoma multiforme (GBM), about 25% of all brain tumors, is only 6 months after surgery. This prognosis has remained the same for a long time. After surgery, the tumor often recurs at the resection site. The contribution of chemotherapy to survival of patients suffering from GBM is uncertain, because of failure in delivery of most of the drugs to the brain. The main reason causing this failure is the existence of the blood-brain barrier (BBB), a highly selective barrier between blood and the brain tissue that keeps components like pathogens or undesired molecules outside the brain. For successful drug delivery, the BBB has to be outwitted. This paper reviews three new methods for drug delivery to the brain and gives insight into their efficacy. The methods that are discussed are delivery of drugs by loaded nanoparticles, delivery of biological products by encapsulated cells and disruption of the BBB induced by focused ultrasound. These techniques are not used in clinic yet, but hopefully contribute to better treatment of malignant brain tumors in the near future.

Keywords: drug delivery, blood-brain barrier, nanoparticles, cell encapsulation, focused ultrasound, brain tumor

Contents

Introduction

Tumors in the brain are responsible for almost 2% of deaths in the Western world. The most common malignant one, about 25% of all brain tumors, is a glioblastoma multiforme (GBM) (Castro et al., 2003). The prognosis of survival of a patient suffering from GBM is only 6 months after surgery, which is usually applied after diagnosis of GBM. Strikingly, this prognosis remained the same for 50 years (Nano et al., 2012), despite much research during this period.

The most used methods nowadays to treat a malignant brain tumor are surgery, radiotherapy and chemotherapy, generally applied as a combination treatment (Anton et al., 2012). These methods are not very effective and are often used not to cure the patient, but only to elongate the patient's life. Removing a brain tumor surgically is very difficult because a border is hardly visible. That is the reason why complete removal rarely succeeds, tumor cells remain at the cutting edge after surgery and form in this way a new tumor at the resection site. Radiotherapy, which is administered after surgery, is used to kill highly proliferative tumor cells and so to inhibit tumor growth. It can prolong the patient's survival, but despite that the prognosis remains poor. For patients older than 70 years, it is suggested that radiotherapy after surgery will not even contribute to a survival benefit (Laperriere et al., 2002). Chemotherapy is often applied in addition to surgery and radiotherapy. It is no curative therapy, but it is used to control tumor growth and to increase the quality of a patient's life (Castro et al., 2003). The actual contribution of chemotherapy to the overall survival in patients suffering from GBM is uncertain (Burton and Prados, 2000).

The main reason why intravenous or oral administration of chemotherapy is not very effective is a failure in the delivery of most drugs to the brain. This is because of the existence of a highly selective barrier between blood and the brain tissue that keeps components like pathogens or undesired molecules outside the brain. This blood-brain barrier (BBB) is built by astrocytes, pericytes, microglia and tight junctions between epithelial cells of blood vessels in the brain (figure 1) and plays a crucial role in regulation of brain metabolism and function (Francis et al., 2003). The tight junctions between the endothelial cells form a strict barrier with a high electrical resistance, preventing ionic and polar (so hydrophilic) substances to enter the brain. Only small (with a molecular mass below 180 Da) lipophilic molecules are able to enter passively (Castro et al., 2003; Yang and Lee, 2012). Besides this structural barrier, there is a physiological barrier called P-glycoprotein, which is also known as multidrug resistance protein and is expressed by cells of the BBB. P-glycoprotein is an ATP-powered efflux system that actively pumps compounds back into the blood and restricts in that way the reception of drugs into the brain (Sharom, 2011).

This restriction of drug delivery to the brain that is caused by the BBB requires a solution. If anticancer drugs can be delivered in the brain efficiently and without limitations, there may be an increased survival prognosis for patients suffering from a malignant brain tumor. In this paper some new techniques for delivering drugs into the brain are discussed. It is possible to 'fool' the BBB by package of drugs into nanoparticles that can cross the BBB. In that way, the tumor receives some kind of therapeutic Trojan horse. In another method, cells that are encapsulated (to prevent rejection) deliver biological products at the original site of the tumor after surgery. It is also possible to disrupt the BBB temporarily by focused ultrasound and thereby to increase the permeability. Hopefully these techniques contribute to a better prognosis of a malignant brain tumor.

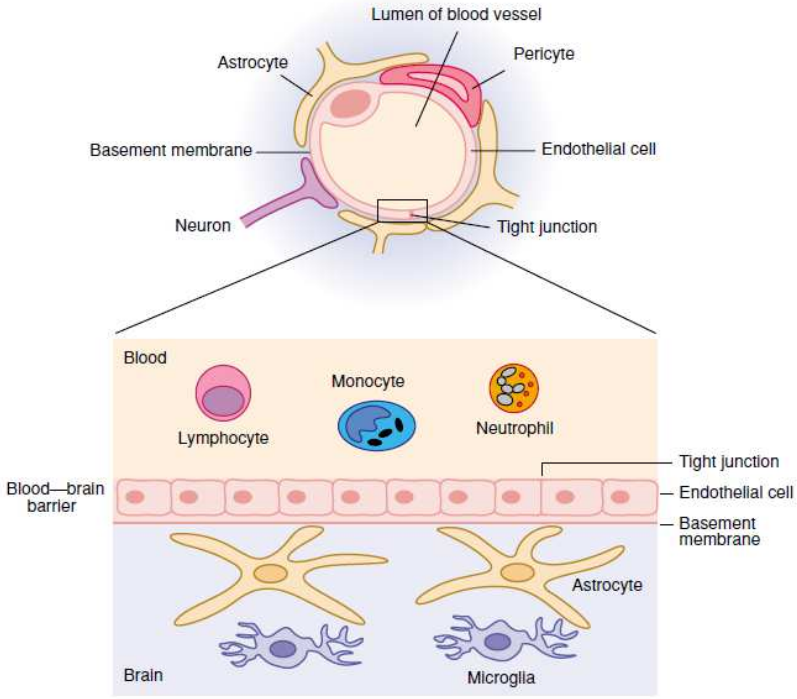

Figure 1
The blood-brain barrier.
The BBB is built by solid tight junctions between endothelial cells of blood vessels in the brain. In this way, it prevents pathogens and other components that can be harmful to the brain from infiltrating (from Francis et al., 2003).

Delivery of drugs by loaded nanoparticles

Lipid nanoparticles

One way to let drugs pass the BBB is to encapsulate them in lipid nanospheres, also named liposomes. Packed in a lipid matrix, substances can more easily enter the brain by passive diffusion. This is because permeability of the BBB is increased by substances with a high lipophilic and non-ionic character and a low molecular weight (Patel et al., 2009). Like a small blessing in disguise, a tumor has a poorly regulated angiogenesis resulting in a leaky vascular system. And because of an inadequate draining system, the interstitial fluid within a tumor is poorly abducted. In this way, substances that have reached the tumor by passive diffusion will not get out easily (Battaglia and Gallarate, 2012).

The last 20 years different kinds of lipid nanoparticles have been developed. As shown in figure 2A, a liposome has a diameter between 10 and 1000 nm. Drugs can be carried either in the interior if they are hydrophilic or within the bilayer in the case of lipophilic drugs. Lipid nanoparticles can be targeted to the tumor site by adhered antibodies specific for properties of tumor cells (Fahmy et al., 2007). An example of such a property is an elevated level of interleukin-13 receptor expression by tumor cells. If liposomes are equipped with antibodies against these receptors, their action is right at the site of the tumor (Madhankumar et al., 2009).

One of the most important properties a nanoparticle must possess is stability and longevity in the blood. That means that excretion of the nanoparticle and interaction with cells of the immune system has to be prevented. This goal can be reached by attaching a hydrophilic polymer called polyethylene glycol (PEG) to the surface of a nanoparticle. According to figure 3, PEG coating causes sterical hindrance for interaction with blood plasma components (Craparo et al., 2011), reduced renal filtration and improved solubility (Junkers, 2010).

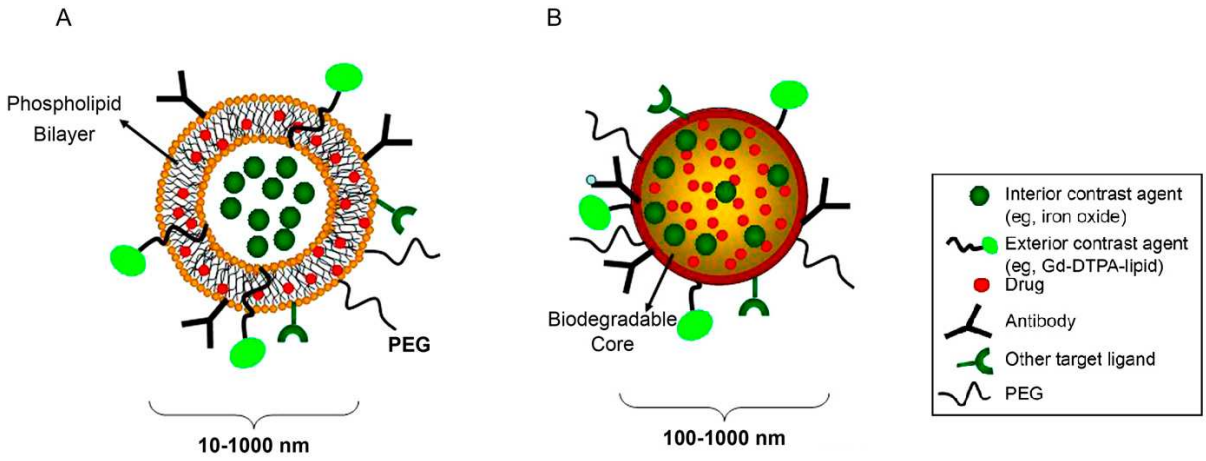

Figure 2 ***Schematic images of a lipid nanoparticle and a polymeric nanoparticle.*** *A liposome (A) can either carry hydrophilic drugs in the interior or lipophilic drugs within the bilayer. A polymeric nanoparticle (B) can carry drugs in the biodegradable core. The hydrophilic polyethylene glycol (PEG) prevents in both kinds of nanoparticles interaction of the nanoparticle with plasma proteins resulting in an enhanced circulating lifetime of the particle (adapted from Fahmy et al., 2007).*

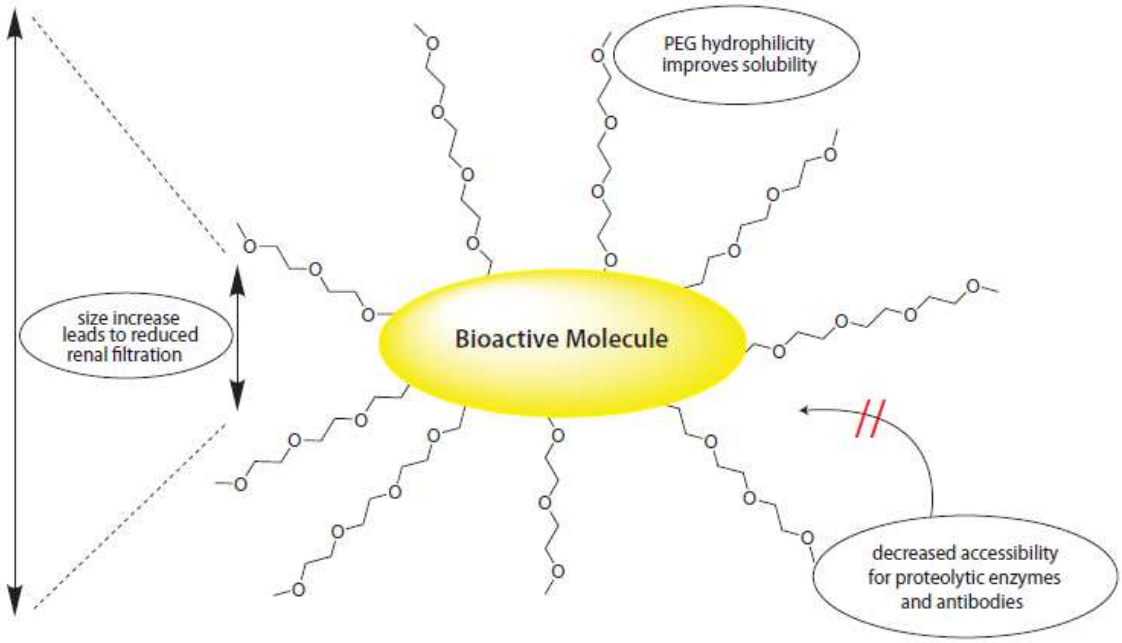

Figure 3 ***Several advantages of PEG coating on a nanoparticle.*** *PEG coating increases circulating lifetime of a nanoparticle, caused by (1) the hydrophilic character which causes an enhanced solubility (2) sterical hindrance, resulting in a decreased accessibility of the particle for blood plasma proteins and (3) a reduction in renal filtration by the increase in size of the particle (from Junkers, 2010).*

Several antitumor drugs (as listed in table 1) that are used as chemotherapy agents are already delivered to the brain by liposomal encapsulation. These studies were often performed in glioma- or GBM-bearing mice. Much research is done to establish the efficiency and safety of delivering these drugs by liposomes, mostly in the last few years. As a commonly used chemotherapy agent, delivery of doxorubicin is intensively investigated. Madhankumar et al. (2009) showed in an *in*

vitro model that doxorubicin loaded liposomes did not injure the endothelial cells of the BBB while still being toxic to astrocytoma cells. Liposomal doxorubicin can also be used in combination with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) to sensitize GBM cells for targeted TRAIL. Treatment with encapsulated doxorubicin before exposure to TRAIL results in an increased rate of TRAIL-induced apoptosis in the GBM cells, but not in normal epithelial cells. This combination therapy causes much greater effects than free (unencapsulated) doxorubicin or TRAIL alone (Guo et al., 2011). Another possibility for combination treatment with doxorubicin and TRAIL is to pack them in the same nanoparticle (figure 4). Bae et al. demonstrated in 2012 the effectiveness of this so called co-delivery of doxorubicin and TRAIL. It resulted in an increased cancer cell toxicity compared to delivery of free TRAIL.

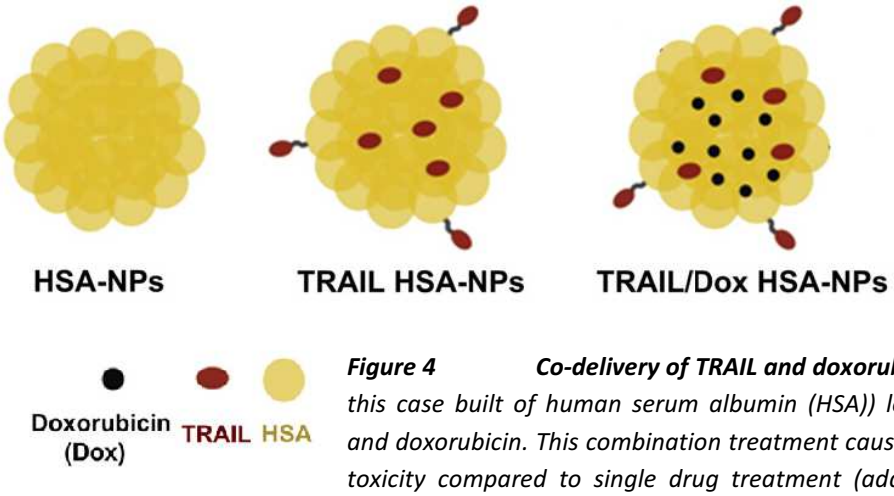

Figure 4 ***Co-delivery of TRAIL and doxorubicin.*** *A nanoparticle (in this case built of human serum albumin (HSA)) loaded with both TRAIL and doxorubicin. This combination treatment causes increased cancer cell toxicity compared to single drug treatment (adapted from Bae et al., 2012).*

Yang et al. reported in 2012 successful delivery of liposomal doxorubicin in glioma-bearing mice. It resulted in an elevated accumulation of the drug at the tumor site and also a tumor-to-normal brain drug ratio that was significantly increased compared to untreated mice. However, free doxorubicin had similar antitumor effects. Liposomal doxorubicin is already available as a cancer medication nowadays, but not yet for treatment of malignant brain tumors. It is known as Caelyx® or Myocet® and it is used to treat breast cancer, ovarium cancer, Kaposi's carcoma and myeloma (1).

Not only doxorubicin, but also other drugs are already delivered in the brain successfully. Verreault et al. showed in 2012 promising results of treatment with liposomal irinotecan in GBM-bearing mice. Treatment with the maximum tolerated dose of 100 mg/kg caused an increase in survival time of 83% compared to untreated animals. The chemotherapy agent topotecan is much more effective in liposomal form. In GBM-bearing mice treated with liposomal topotecan, tumor growth was slowed down and survival rate was significantly higher than in animals that received treatment with the free form (Serwer et al., 2011). That not only doxorubicin but also paclitaxel is very effective in co-delivery with TRAIL, showed Sun et al. in 2012. They treated glioma-bearing mice with liposomes containing both paclitaxel and TRAIL, resulting in a more evident and widespread apoptosis of glioma cells and a significantly increased survival time compared to single medication therapy.

Table 1 Antitumor drugs delivered in the brain by liposomal encapsulation.

Antitumor drug delivered by liposomes	**Study**	**Shown in**
Doxorubicin	Madhankumar et al., 2009 Agarwal et al., 2011 Verreault et al., 2011 Yang et al., 2012	A model of the BBB Glioma-bearing mice GBM-bearing mice Glioma-bearing mice
Irinotecan	Verreault et al., 2011 Verreault et al., 2012	GBM-bearing mice GBM-bearing mice
Imipramine Blue	Munson et al., 2012	Glioma-bearing rodents
Vincristine	Verreault et al., 2011	GBM-bearing mice
Topotecan	Serwer et al., 2011	GBM-bearing mice
Paclitaxel	Sun et al., 2012	Glioma-bearing mice
Cytarabine	Passarin et al., 2010	One astrocytoma patient
CCNU (lomustine)	Yimam et al., 2006	Glioma-bearing rats

Polymeric nanoparticles

It is also possible to prepare nanoparticles made of non-biological origin. In contrast with lipid nanoparticles, there is no limitation in drug loading caused by hydrophobic or lipophobic properties. As seen in figure 2B, drugs can simply be packed within the particle. It was for the first time reported in 1995 that polymeric nanoparticles crossed the BBB after intravenous injection (Kreuter, 2012). A mechanism that is suggested to be responsible for crossing is transcytosis after receptor-mediated endocytosis. This endocytosis is attained by attaching a ligand to the nanoparticle that binds to a receptor that is expressed at the surface of endothelial cells of the BBB (Kreuter, 2012). An example of such a ligand is transferrin that binds its receptor at the BBB and thereby mediates transcytosis of the nanoparticle (Bae et al., 2012). Just like lipid nanoparticles, are most polymeric nanoparticles equipped with a PEG coating, preventing interaction of the particle with blood plasma components (Fahmy et al., 2007; Craparo et al., 2011).

An example of beneficial effects reached by loaded polymeric nanoparticles is given by López-Donaire et al. in 2009. They treated human GBM cells and normal human fibroblasts with OAG, a synthetic antimitotic drug, which was encapsulated in a polymeric shell. These nanoparticles showed dose dependent selectivity for the GBM cells but not for normal fibroblasts. Treatment with OAG containing nanoparticles did not affect the fibroblasts; however, the GBM cells expressed a significantly decreased viability.

The transport rate of a nanoparticle can be extended by an amphiphilic design. In contrast to liposomes, polymeric nanoparticles with an amphiphilic shell have fewer restrictions in transporting drugs (López-Donaire et al., 2012). And because the core is biodegradable by enzymatic surface erosion, a controlled release of the drug is possible as shown by Jiang et al. in 2011. Effects of paclitaxel (a chemotherapy agent) encapsulated in amphilic biodegradable polymeric nanoparticles were compared to those of injection of free paclitaxel. An 80% release occurred after less than two hours in the case of free paclitaxel, but only after 24 hours in case of the encapsulated form. They also reported a median survival time that was significantly increased in glioma-bearing mice treated with the encapsulated form compared to the animals treated with the free form.

Rate of cellular delivery of drugs can be increased by modifying the surface of the polymeric nanoparticle using a nonionic surfactant. A study of Tahara et al. (2011) in human GBM cells describes a cellular uptake of doxorubicin delivered by nanoparticles containing the surfactant P80 that is about three times higher than P80 lacking ones. A possible mechanism behind this increase suggested by the authors is that the surfactant P80 makes the cell membrane more fluid, resulting in a higher permeability and so an increased rate of endocytosis through the cell membrane. Beneficial effects of P80 nanoparticles containing doxorubicin are also seen *in vivo*. Wohlfart et al. (2011) showed in GBM-bearing rats a decreased tumor size and proliferation rate after treatment with P80 containing nanoparticles compared to treatment with free doxorubicin.

It is suggested that the beneficial antitumor effects of polymeric nanoparticles can be augmented by using them in combination with thermotherapy. In this method, particles are equipped with magnetic iron-oxide in the core, after which they are planted directly into the tumor and subsequently heated by an electromagnetic field (Maier-Hauff et al., 2011). Ito et al., (2003) demonstrated a strong inhibition of tumor growth in glioma-bearing rats after thermotherapy of implanted magnetic nanoparticles. In a study of Jordan et al. in 2006 it is also shown that thermotherapy with magnetic nanoparticles causes a significant increase of survival of glioma-bearing rats. However, this was only reached by particles coated with an aminosilane shell, not by those with a carboxydextran shell. In a clinical study of Maier-Hauff et al. from 2007, the safety of using this method was evaluated in patients suffering from GBM. There were no striking complications or side effects found. Therefore, the researchers concluded that the use of magnetic nanoparticles followed by thermotherapy is a safe and promising method for treating brain tumors. However, the same group of researchers found in 2011 no significant beneficial effect of treatment with magnetic particles on survival of GBM patients. The only beneficial outcome was a slight diminution of tumor volume. What they did find, was a confirmation of the clinical safety of this method in patients (Maier-Hauff et al., 2011).

Delivery of biological products by encapsulated cells (ECs)

Another strategy to treat malignant brain tumors that is intensively investigated is cell encapsulation. Instead of drugs that are being encapsulated in either lipid or polymeric nanoparticles, are in this case cells packed in a protective microcapsule. Because these microcapsules are placed directly into the brain after surgery, there are no tricks needed to let them pass across the BBB.

Cells used in this method are xenogeneic and genetically modified to express a certain recombinant protein. They are packed in a spherical capsule with a diameter of 100-1000 µm (Visted and Lund-Johansen, 2003). The outer material is often made of alginate, a natural polysaccharide derived from brown seaweed, because it is a long-term stable and viable compound (Visted et al., 2001). This shell plays an important role in protecting the ECs from being cleaned up by the immune system of the receiver. It is semipermeable so oxygen and nutrients can reach the cells and the protein expressed by the cells can be transferred out of the microcapsule (figure 5). In this way, the ECs serve like a biological depot of therapeutic agents that ideally control tumor growth (Visted and Lund-Johansen, 2003).

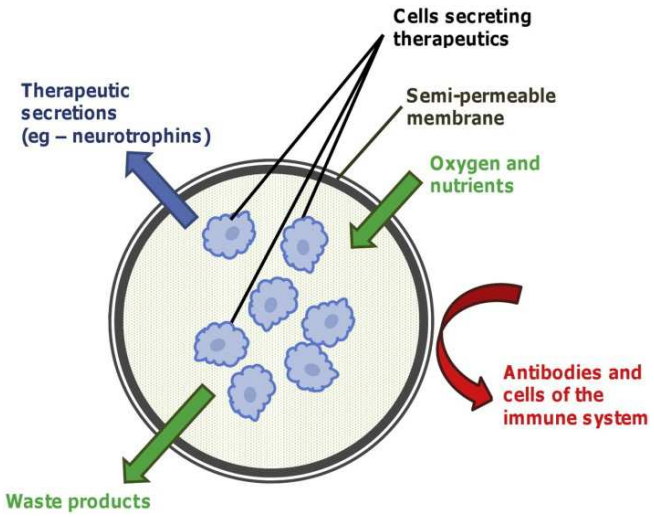

Figure 5
Schematic image of encapsulated cells. *Cells that are excreting therapeutic agents are packed in a microcapsule that protects the cells from being cleaned up by cells of the immune system. The membrane of this capsule is semipermeable, so oxygen and nutrients can be taken up and the agents can be excreted (from Zanin et al., 2012).*

An example of a biological product delivered by ECs is endostatin, an inhibitor of angiogenesis. It is suggested that this process of angiogenesis contributes to the malignancy of brain tumors (Joki et al., 2001). Angiogenesis is a comprehensible target in fighting brain tumors because they show high angiogenic activity, seen as an elevated production of vascular endothelial growth factor (VEGF) by tumor cells (Polivka et al., 2012). In 2001 Joki et al. demonstrated that encapsulated hamster cells modified to produce endostatin had beneficial effects on tumor growth in glioma-bearing nude mice. Treatment with these ECs resulted in a decrease in tumor weight of nearly 75% compared to treatment with unmodified ECs that did not produce endostatin.

Cells that are being used for encapsulation must have two important properties: the chance of rejection has to be minimal and they have to be easily genetically modifiable. Cells that qualify these requirements are stem cells. They are hypoimmunogenic and genetic modification is well possible (Goren et al., 2010). It is also known about stem cells that they migrate to the site of a tumor driven by chemo-attractant cytokines (Eskandary et al., 2011). However, this is a less relevant property concerning cell encapsulation, because cells cannot leave the microcapsule. In 2010 Goren et al. studied the therapeutic opportunities of encapsulated mesenchymal stem cells. In immunogenicity experiments, they observed a cytokine release in response to the encapsulated stem cells that was only a third of the response to other encapsulated cell lines. In the study of Goren et al., stem cells were programmed to produce hemopexin-like protein (HPX), an inhibitor of angiogenesis. Treatment with these ECs provided an astonishing decrease in tumor volume of 89% in GBM-bearing mice compared to treatment with empty microcapsules or ECs producing only a fluorescent protein.

In most studies the outer shell of microcapsules is built of alginate (figure 6). However, also stem cells packed in a synthetic biodegradable extracellular matrix are demonstrated to be effective. Kauer et al. (2011) examined the therapeutic efficiency of stem cells that were modified to produce secretable TRAIL that selectively induces apoptosis in tumor cells. They found that GBM cell viability and tumor volumes in mice treated with TRAIL producing ECs had decimated after 24 hours compared to treatment with ECs producing a fluorescent protein. In addition, they observed a much higher activity of caspase-3/7 in TRAIL treated mice, indicating an elevated rate of apoptosis of GBM cells.

Malignant brain tumors cannot only be fought by ECs that produce proteins; it is also possible to use ECs that excrete genes by retroviral vectors. Martinet et al. (2003) encapsulated cells

that produce retroviral vectors carrying a suicide gene which, according to its name, induces apoptosis in other cells. They investigated the efficacy in GBM-bearing rats. In this model, the necrotic tumor volume was significantly higher after treatment with ECs that excreted the suicide gene compared to treatment with a free viral vector or with an empty microcapsule.

Because tumor cells are often highly expressing particular molecules like certain receptors, delivery of genes can be specifically targeted. An example of such a receptor that is highly expressed by gliomas is the somatostatin receptor. Treating human GBM cells with a retroviral vector containing a somatostatin inhibiting factor can result in control of GBM cell growth (Lécolle et al., 2012). It is also possible that tumors do not show elevated but reduced levels of expression of certain factors. Ligon et al. (1997) discovered a new gene product, RIG, which was down-regulated 10-fold in GBM cells. Knowing this, therapy can be directed to the desired site of action.

Cells used for studying encapsulation are often xenogeneic. Although the cells are packed in a microcapsule, a foreign body reaction of the host is hardly avoidable. Molecules with a small molecular weight like cytokines and reactive oxygen species can pass the microcapsule. These products excreted by inflammatory cells of the host can earnestly impair the ECs, resulting in dysfunctional or even dead ECs (Orive et al., 2004). Also the microcapsule material is susceptible of an immune response. Robitaille et al. investigated in 2005 the immunogenicity of empty alginate microcapsules after peritoneal implantation. They reported a significantly increased gene expression of the cytokines TNF-α, IL-1β, IL-6 and TNF-β after implantation of microcapsules compared to saline injection. After implantation of microcapsules, a significant higher number of inflammatory cells like macrophages, eosinophiles and lymphocytes were present in the peritoneal fluid. It is shown that the inflammatory response to ECs lasts for a few weeks (Orive et al., 2006). A possible way to minimize this foreign body reaction is to design an encapsulation delivery system that blocks the CD154-CD40 pathway. Blocking of this pathway inhibits harmful actions of a cellular immune response and increases the probability of long-term survival of ECs (Blanco-Bose et al., 2006).

A lot of research takes place nowadays to augment the biocompatibility and safety of both microcapsule material and cell lines that are suitable for cell encapsulation.

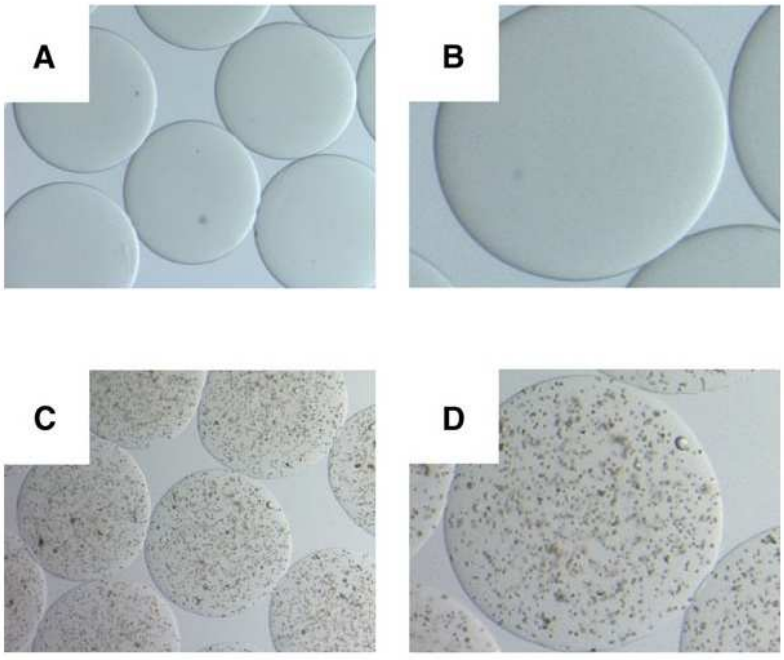

Figure 6
Eukaryotic cells encapsulated in alginate microcapsules.
Empty microcapsules (A-B) and microcapsules containing IB3-1 cells, an immortalized cell line of bronchial epithelia cells (C-D) (from Mazzitelli et al., 2011).

Disruption of the blood-brain barrier by focused ultrasound

At first sight maybe a less elegant way to deliver drugs to the brain is to simply disrupt the BBB and thereby temporarily increasing the permeability for macromolecular agents. In that way a higher rate of drug delivery can be achieved without increasing the dose. In a method called focused ultrasound (FUS), repeating pulses of ultrasound waves of low frequency are provided transcranially, usually for 20 to 30 seconds. Because of the low frequency of the waves, tissue damage is minimal. This technique is often applied in combination with gas microbubbles with a diameter of 1-5 µm (figure 7). These microbubbles are administered intravenously beforehand, resulting in an even more diminished threshold for BBB disruption (Etame et al., 2012). BBB disruption by FUS is achieved by disintegration of tight junctions between the endothelial cells. This disintegration manifests as a reduced expression of tight junction component proteins occludin, claudin-5 and ZO-1, which results in an increased permeability (Sheikov et al., 2011). MRI is often used to localize the exact area of interest for a guided, focally applied disruption of the BBB (Etame et al., 2012).

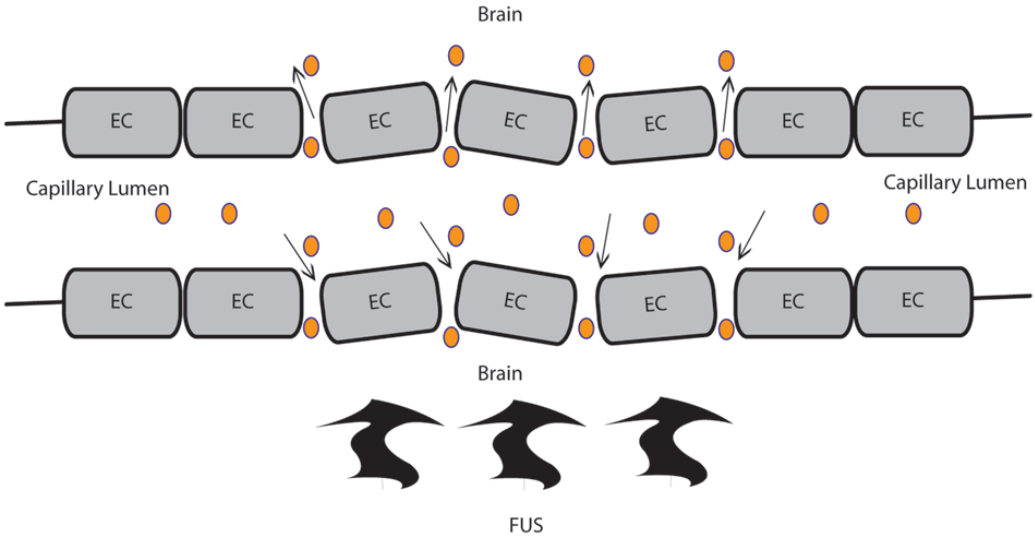

Figure 7 ***Disruption of the blood-brain barrier by focused ultrasound.*** *The waves of the ultrasound (FUS) let microbubbles (orange) oscillate, thereby causing disruption of the structure of the BBB resulting in an increased permeability (from Etame et al., 2012).*

Advantages of FUS are that it is a noninvasive method and that the ultrasound can be applied focally. Increased permeability of the BBB can also be accomplished by application of osmotic agents like bradykinin or mannitol, which cause vasodilatation and shrinkage of the endothelial cells in the BBB. These alterations result in a widening of the tight junctions and thereby an increased permeability of the BBB (Rapoport, 2000). However, administration of these agents occurs intra-arterial so a proper control of the effects is limited. It causes unfocusal and widespread disruption of the BBB whereby the brain is not protected against systemic toxins (Etame et al., 2012).

In animal models, FUS has been shown as a promising method. Yang and Horng (2011) injected glioma-bearing rats with Evans blue with or without sonification with transcranial focused ultrasound. After sonification, BBB permeability was significantly enhanced as seen by an increased Evans blue accumulation and extravasation in the brain (figure 8). That FUS is also successful in bigger, more human-like animals showed McDannold et al. in 2012. They evaluated the efficiency

and safety of using FUS to disrupt the BBB in rhesus macaques. They found a safety window of parameters (like duration and acoustic pressure) in which successful delivery of a contrast agent after BBB disruption could occur without brain tissue damage. FUS was applied for several weeks within visual areas of the brain, followed by complex visual tasks. The animals showed no deficiencies in these experiments. Not only contrast agents can be delivered successfully, but also the commonly used chemotherapy agent BCNU (Ting et al., 2012). Glioma-bearing rats were treated with BCNU and did or did not receive FUS. In animals that received BCNU and FUS, shrinkage of tumor volume of 10% was visible compared to animals receiving no FUS and 15% compared to glioma-bearing control animals.

Effects of several parameters like duration, frequency and pulse length of sonification with ultrasound on permeability of the BBB are being intensively investigated for an optimization of the procedure (O'Reilly et al., 2011; McDannold et al., 2008).

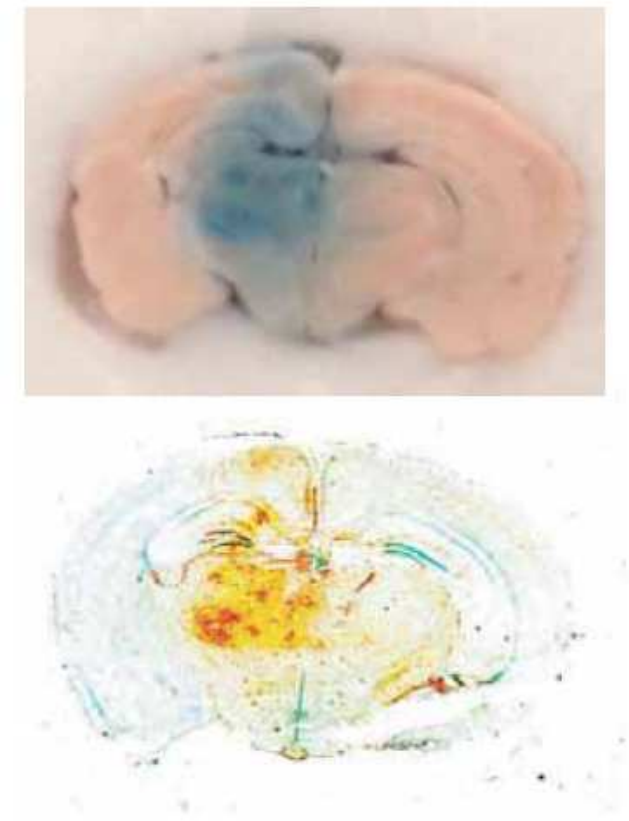

Figure 8
Extravasation of pigment after FUS induced disruption of the BBB.
A post mortem picture of a mouse brain, showing extravasation of, in this case, Trypan blue (top). The same brain slice showing antibody staining of extravasation (bottom). These pictures verify disruption of the BBB by FUS (from Kinoshita et al., 2006).

Discussion

The problem of successful drug delivery to the brain is caused by the strict selectivity of the BBB. This barrier can be circumvented by packing drugs into nanoparticles that can cross the BBB. The brain receives in this way a Trojan horse loaded with antitumor agent. Delivery of liposomal chemotherapy to the brains of glioma-/GBM-bearing mice has been proven successful (Agarwal et al., 2011; Verreault et al., 2011; Yang et al., 2012). One chemotherapy agent, doxorubicin, is even available in liposomal form already (1).

Although many beneficial results have already been achieved in animal models, there is still a lot of research going on to the biocompatibility and targeting of nanoparticles. These are the two

major challenges that still remain. Nanoparticles have to be targeted specifically to tumor cells, preventing that cytostatic or cytotoxic drugs are delivered to healthy brain tissue. Also, the nanoparticle itself has to be as little toxic as possible. This requirement is less relevant when using lipid nanoparticles, because they consist of lipids which occur naturally in the body (Yang, 2010). Biocompatibility plays a more important role in investigating delivery by polymeric nanoparticles. The chance of a foreign body reaction is decreased by attachment of PEG molecules to the surface of a nanoparticle, because PEG coating prevents interaction with blood plasma components (Craparo et al., 2011). However, research remains to be done to the toxicity of the polymeric packaging.
Another important challenge is controlled release of the antitumor drug. A nanoparticle has to be loaded with enough drugs to effectuate a therapeutic effect, but the risk of toxicity of the released dose has to be minimized if disintegration occurs too quickly. A balance needs to be found between a high therapeutic effect and a low toxicity. A controlled release is easier to reach when using a polymeric nanoparticle. Because this is not a hollow sphere like a liposome, the nanoparticle can be designed in a way that it exists of multiple layers. If drugs are equally distributed in several compartments, drug release occurs incrementally.

Lifetime of the nanoparticles and the drug they carry in the brain is another subject of research. This can be increased by a PEG coating, which not only prevents a foreign body, but also reduces renal filtration (Craparo et al., 2011). An increased lifetime can also be achieved by loading a higher drug dose in a nanoparticle. However, to augment loading capacity, a size increase is hardly avoidable and a greater nanoparticle may trigger the immune response even more. So it remains a challenge to increase loading capacity without increasing the size.

A method that requires fewer skills to circumvent the BBB is implantation of little depots of biological antitumor agents. These can be excreted by ECs that are genetically modified to express certain recombinant proteins. Microcapsules are placed at the site where a brain tumor is removed surgically. Therefore it is, in contrast to delivery by nanoparticles, an invasive method. But a malignant brain tumor is often resected anyway, so extra surgery is not necessary. However, it becomes a problem when the cells are not functioning well in excreting antitumor agents, or when the cells die in their capsule. The only way to remove them then, is by surgery. Because of the encapsulation, they cannot be cleaned up by the immune system of the host.

Research to this method is often focused on immunogenicity of both the microcapsule material and the ECs. The microcapsule is often made of alginate, a natural polysaccharide derived from brown seaweed. But implantation of microcapsules rarely happens without an elevated expression of cytokines and an increased appearance of inflammatory cells (Robitaille et al., 2005). The cells that are used for encapsulation are xenogeneic, so steps have to be taken to avoid a severe immune response with destruction of the microcapsule as a result. A solution is suggested by Goren et al. in 2010. They used mesenchymal stem cells because of their hypoimmunogenic properties. These cells provoked a three-fold lower cytokine response compared to encapsulation of other cell lines. A way to exclude an immune response of the host is to isolate some of the patient's own stem cells, modify and culture them and encapsulate them. If this is not possible for some reason, HLA-matching human cells can be used from another person. However, it may be an ethically sore question to genetically modify human stem cells.

A controlled release of therapeutic proteins by ECs is very well possible, because they are newly synthesized at the site of the tumor. These proteins do not have to be transported a long way through the body and they can be directed by antibodies against the tumor cells. A problem arises

when cells escape from their microcapsule. Healthy cells outside the brain may be impaired when they are exposed to the recombinant protein. Microcapsules have to be long-term stable and indestructible by inflammatory cells. The immunogenicity of the microcapsule material as well as the ECs has to be as low as possible to prevent rejection.

FUS is a noninvasive method that allows drugs to enter the brain without interaction with the BBB. A higher rate of drug delivery can be reached without increasing the dose. Chemotherapy agent delivery to the brain is demonstrated in rodent tumor models (Ting et al., 2012). Lipid nanoparticles loaded with doxorubicin were delivered successfully after FUS induced disruption of the BBB (Treat et al., 2012). FUS has also been proven safely and successfully in swine (Wei et al., 2012) and rhesus macaques (McDannold et al., 2012). The acoustic force that is required for disruption of the BBB is over 100-fold smaller than the force that causes severe brain tissue damage, so there is no risk of long-term injury (Hynynen, 2008).

Because the ultrasound can be applied focally, there is no widespread disruption of the BBB but only at the location where antitumor drugs have to carry out their effect. One issue that may be a challenge is control of duration of BBB disruption. The BBB normally exists to prevent entry of undesired molecules that can harm metabolism in the brain. If this barrier is 'open' for a longer time than is required to deliver drugs, physiological processes in the brain can be disturbed. Sheikov et al. demonstrated in 2011 that leakage of substances lasted up to four hours after application of FUS.

Apart from this challenge, FUS is a promising tool to bring drugs into the brain. Parameters like duration, frequency and pulse length of sonification have to be optimized in clinical experiments. For example, humans have a much thicker skull than rodents so localization and sonification of the target area will be more difficult than in animal models (Kinoshita, 2006).

Concluding remarks

This paper has reviewed three new methods to deliver drugs to the brain: delivery of drugs by loaded nanoparticles, delivery of biological products by ECs and disruption of the BBB by FUS. In all of these methods, the BBB is outwitted somehow. The two major challenges in delivering drugs by loaded nanoparticles are regulation of a controlled release and biocompatibility of the nanoparticle itself. Most research is done in rodents, with varying results. I think it will take a long time before this method will be used in clinic. Too many risk factors are still remaining, like a proper control of release and toxicity to brain tissue, the last one especially in the use of polymeric nanoparticles. Cell encapsulation is an elegant technique to deliver anticancer agents to a brain tumor. ECs are capable of a dosed release of biological products that induce apoptosis in cancer cells. An obstacle that remains is that these ECs can only be removed invasively if necessary. Before this method can be investigated in clinical trials, research has to focus on designing microcapsules in a way that both the microcapsule and the ECs are inaccessible for inflammatory cells and cytokines of the host. In my opinion, FUS is a highly promising technique which is closest to being used in clinic. It has been proven a safe and efficient tool to deliver drugs to the brain. Most research occurred in rodent tumor models, so first beneficial results need to be confirmed in models that are more representative of humans before clinical trials can be performed to optimize sonification parameters.

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