Convection-enhanced delivery of nanocarriers for the treatment of brain tumors

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Abstract
Primary brain tumors have a significant infiltrative capacity as their reappearance after resection usually occurs within 2 cm of the tumor margin. Local delivery method such as Convection-Enhanced Delivery (CED) has been introduced to avoid this recurrence by delivering active molecules via positive-pressure methods. For an efficient infusion, the distribution volume of the drug has to be optimized while avoiding backflow, since this is responsible for side effects and a reduction of therapeutic efficacy. The encapsulation of the drug infused in nanosized structures can be considered, which would lead to a reduction of both toxicity of the treatment and infusion time during CED. In the present review, we will firstly discuss the technical approach of CED with regard to catheter design and brain characteristics; secondly, we will describe the ‘ideal’ nanocarrier in terms of size, surface properties, and interaction with the extracellular matrix for optimal diffusion in the brain parenchyma. We also discuss preclinical and clinical applications of this new method.

1. Introduction
The incidence of primary central nervous system tumors (PCNST) is increasing, especially in the younger population as it represents the second cause of cancer death in adults less than 35 years of age. In the United States, about 1–2% of the population is affected and consequently suffers from profound and progressive mortality, as evidenced from the 20,500 new brain cancer cases and the 12,740 deaths estimated in 2007 [1]. A French study has described an incidence of 15.8/100,000 persons per year affected by PCNST [2]. Among the brain tumors, half originate from glial cells and are thus classified as gliomas, and more than three quarters of all gliomas are astrocytomas. Astrocytomas constitute a heterogeneous group of tumors that range from low grade to the most aggressive, glioblastoma multiforme (GBM), based on histopathological classification (from grade I to IV WHO – World Health Organisation). GBM differs from the other cancers by its diffuse invasion of the surrounding normal tissue and its recurrence after all forms of therapy. The overall incidence of malignant glioma grades III and IV (WHO) in industrialised nations is 5–11 new cases per 100,000 people per year [3].

Conventional therapy includes surgical biopsy for pathological diagnosis and if it is possible, the first treatment is tumor resection, followed by fractioned external beam radiotherapy and systemic or oral chemotherapy [4–6]. Despite these treatments, the prognosis for patients with glioblastoma has remained largely unchanged over the last three decades. Stupp et al. described a median survival time of 14.6 months for patients treated with radiotherapy plus temozolomide which is the reference chemotherapy, and 12.1 months with radiotherapy alone [7,8]. The difficulty with treating brain tumors is the effective delivery of therapeutic agent to the tumor as well as to infiltrate cells that are not located in the tumor bed. If these outlying tissues are not treated, the tumor will reappear.

Because of the presence of the blood brain barrier (BBB), the failure of conventional systemic drug delivery for gliomas has motivated more direct approaches [9–11]. An alternative treatment is the local administration of the agent from a degradable or non-degradable polymer delivery system implanted at the site of the disease [12,13]. Although this technique presents some advantages such as sustained and controlled drug release, it is also characterized by poor drug penetration and drug dosage limited by the implant size.

Recently, it was shown that fluid convection, established by maintaining a pressure gradient during interstitial infusion, can supplement simple diffusion to enhance the distribution of small and large molecules in brain and tumor tissue. This technique called Convection-Enhanced Delivery (CED) was proposed and introduced by researchers from the US National Institutes of Health (NIH) by the early 1990s to deliver drugs that would not cross the
BBB and that would be too large to diffuse effectively over required distances [14]. In this case, in situ drug concentrations can be significantly greater than those achieved by systemic administration [15,16]. This technique allows the local delivery of a wide range of substances like conventional chemotherapeutic agents [17–19], monoclonal antibodies [20,21], targeted toxins [22–24], other proteins [25], viruses [26,27], and nanocarriers [28–30]. During the first decade after the NIH researchers founded this analytical model of drug distribution, the results of several computer simulations that had been conducted according to realistic suppositions were also published, revealing encouraging results [31].

For the effective functioning of CED, the activity of the anti-cancer agent has to be considered but the technical drug delivery approach appears to be a critical parameter. In fact, a uniform distribution of a truly effective agent in tumors will ultimately influence the therapeutic efficacy. This is the reason why experimental protocols have to take care of different parameters proper to CED injection.

Moreover, properties of each infusate have to be considered. Nanocarriers like polymer and lipid nanoparticles, micelles, liposomes, and dendrimers are often used to vehicle some drugs that are very sensitive, toxic, or hydrophobic, or in order to target specific organs [32]. Such nanoparticulate systems have some inner properties that have to be considered for optimal convection delivery. This review aims at firstly discussing, the technical approach of CED with regard to the materials used and the model investigated. Then the review will focus on specific properties of the infusate limiting our discussion to the use of non-viral nanocarriers such as liposomes, nanoparticles, dendrimers and micelles. Finally, animal and human trials which deliver nanocarriers in CED for therapeutic applications will be explored.

2. Technical approach of convection-enhanced delivery

2.1. Convection-enhanced delivery mechanism

Convection-enhanced delivery (CED) is a novel approach to deliver drugs into brain tissue and is defined as the continuous injection of a therapeutic fluid agent under positive pressure. This recent technique using convection or ‘bulk flow’ was proposed to supplement simple diffusion which characterizes local intracerebral delivery by stereotactic injections (Fig. 1). Stereotaxy is the methodology involved in the three-dimensional localization of structures within the brain, based on diagnostic image information, and the use of stereotactic frame to reach these points. Horsley and Clarke described the first use of an apparatus for neurophysiological animal experiments in 1906 and named their technique ‘stereotactic’ (Greek: stereo = three-dimensional (3D), taxis = to move toward) [33]. The first human stereotactic apparatus was described 40 years later by Spiegel and Wycis [34]. A stereotactic head frame is based on a 3D coordinate system consisting of three orthogonal planes, which are related to external skull points. Stereotaxy can be used to approach deep-seated brain lesions with a probe, a cannula or a high energy ionizing radiation beam [35].

Diffusion is defined as a type of passive transport (non-energy requiring) involving the movement of small molecules from an area where they are highly concentrated to an area where they are less concentrated. The diffusion of a compound in a given tissue depends mainly on 2 parameters: the free concentration gradient and the diffusivity of this compound in the tissue. With the classic diffusion technique, high molecular weight compounds (neurotoxic factors, antibodies, growth factors, enzymes) are not able to diffuse over large distances and drug distribution is very limited, thus reducing the treatment efficacy of neurological disorders [36]. For example, 3 days can be necessary for an IgG to diffuse 1 mm from its delivery site. Moreover, small drugs with good diffusion characteristics can be metabolized or quickly eliminated by capillaries reducing their diffusion in surrounding tissues [37,38]. On the contrary, CED is powered by bulk flow kinetics which occur secondary to pressure gradients. Convection, which can be used to supplement diffusion, relies on a simple pressure gradient, and is independent of molecular weight. In practice, drugs are delivered continuously via a catheter connected to a syringe pump, thus enabling the distribution of large volumes of high drug concentrations with minimum systemic toxicity (Fig. 1).

During CED, diffusion and convection take place simultaneously (Fig. 2). The phenomenon of diffusion is strictly dependent on a concentration gradient on the one hand and on the diffusivity of the infusate in a specific tissue on the other hand. Diffusion occurs all the time, but is rigorously dependent on the nature of the infusate. By CED, the agent is mainly distributed within the interstitial spaces of the tissue by convection itself. The bulk flow, which is strictly dependent on the pressure gradient, occurs throughout the establishment of the pressure gradient.

![Fig. 1. Stereotactic injection in rat brain by classic diffusion method (A) versus convection-enhanced delivery (B). Infusate diffusivity is predominant in CED techniques as large volume of distribution (Vd) can be achieved compared to those obtained after a classic diffusion method.](image-url)
With regard to the shape of drug distribution, the diffusion process leads to a gradient of concentration which decreases exponentially from the point of injection toward surrounding tissues. The convection process allows the obtention of a higher concentration over a longer distance (with reference to the point of injection); the concentration profile is constant during infusion and decreases in an abrupt way at the end of the process (Fig. 2). By using convection to supplement simple diffusion, an enhanced distribution of small and large molecules can be obtained in the brain while achieving drug concentration greater than systemic levels [14]. High-flow microinfusion, like the CED technique, offers the potential of treating much larger volumes of brain tissue than is possible with low-flow delivery methods based on diffusion. Morrison et al. showed that a 12-h high-flow microinfusion of a designed macromolecule would provide 5 to 10-fold increases in volume over low-flow infusion, and provided total treatment volumes superior to 10 cm³ [39].

Some experimental approaches can be considered to follow the distribution of an infusate in a brain structure. Chen et al. compared the distribution and pressure profiles obtained after CED of small molecular weight infusates (Mw = 570–700) in pig animal models on the one hand, and in low-concentration agarose gels used as experimental models on the other hand [40]. Even though agarose gels are inert, non-perfused, homogeneous and isotropic, the ratios between distribution volumes (Vd) and infusate volumes (Vi) were in the same range of order and equivalent to 10 and 7.1 for 0.6% agarose gel and pig brain, respectively. In addition, the infusion pressure of the gel at this concentration was typically close to that found in pig brain (10–20 mmHg). They concluded that a 0.6% agarose gel was a useful in vitro model to mimic the global behavior of real infusion in pig brains, especially when MR imaging was not available. Linninger et al. went further and introduced an innovative mathematical method to calculate the impact of individual tissue properties on molecular transport in CED [31]. This computer-aided methodology allows the reconstruction of the brain geometry for a specific patient, and gives an estimation of heterogeneous anisotropic transport properties by diffusion tensor imaging (DTI) data. Finally, this technique can predict the drug distribution based on rigorous transport principles.

2.2. The key parameters

CED is a complex process that is governed by many parameters. This review aimed at listing the technical parameters directly linked to delivery by convection and especially to the volume of distribution (Vd), and the control of the backflow mechanism.

2.2.1. Regions of the brain

The different regions of the brain are not equivalent in terms of molecular transport mechanism because of a distinct internal structure. Gray matter is mainly composed of the somas of neurons and glial cells. The effective diffusivity in gray matter is almost the same in all directions, and the transport in the gray matter is qualified as isotropic (Fig. 3). White matter contains bundles of axons leading to the peripheral nervous system. The permeability of the white matter changes in accordance with directional alignment and density of axonal fibers. Hence, white matter diffusion is anisotropic. A widespread of agents can be achieved in both white and gray matter, but white matter exhibits a greater ability to accommodate infusate because it is more densely packed and there is less extracellular space [14,41,42]. Because rat brains have very limited white matter in their structure, this parameter would be better studied in larger animal models like primates, dogs and of course, in humans.

Moreover, most studies defining CED parameters have been carried out on normal brain tissue and not in a tumor environment. Vavra et al. showed that distribution in a brain tumor model was a parameter not to be ignored since interstitial fluid pressure is higher in intracranial tumors [15] and may be responsible for the asymmetric distribution of drugs in glioma-bearing rats. Moreover,
the presence of oedemas, often observed in brain cancer, can reduce the flow of the infused agent. When infused into a tumor, which means into tissue where the hydraulic conductivity and extracellular fraction may change radically, liposomes are characterized by an irregular distribution with the presence of nanocarriers into the encapsulated tumor margins [43]. Globally, there is a lack of knowledge about the distribution of infusate in the brain tumor environment.

2.2.2. Catheter placement

Catheter placement is very important for several reasons and especially for preventing the occurrence of backflow (or leakage-back). Backflow can lead to the spreading of the agent into regions of the brain where it is not intended to be and, possibly, to a diminution of the dose otherwise needed within the target tissues. The problem can be particularly acute in cortical infusions, when backflow of the agent along the insertion track and into the subarachnoid space can occur, with the subsequent widespread distribution of the agent via the circulating cerebrospinal fluid (CSF) (Fig. 3). Raghavan et al. described an example which illustrates the leakage of an infused agent into the subarachnoid space via backflow into the catheter during the infusion. A 0.85 mm-diameter catheter was inserted through a burr hole into an in vivo pig brain to a depth of 14 mm from the cortical surface. A Gd-DTPA in water solution (1:200) was infused at 5 ml/min and three-dimensional MR images were obtained to analyze the dispersion of the Gd marker. Images obtained after 32 min of infusion showed evidence that the infused agent had mostly leaked into the subarachnoid space and was widely dispersed along the contours of the cortex, whereas little distribution into the white matter had occurred [44]. Lidar et al. described this phenomenon with the infusion of Taxol™ in patients with GBM. Leakage of the drug into the CSF was described because of a bad catheter location, and was responsible for side effects such as chemical meningitis. Catheter location is therefore highly important as it can cause complications.

Catheter placement also depends on the Vd of a studied infusate. Linninger et al. ask the question: “which injection site is best for maximizing the drug distribution in a specific target site without causing side effects and excessive tissue stress damage?” To answer this, they aimed at targeting the human caudate nucleus (gray matter), and studied the final Vd at 4 weeks, for four different catheter locations: the thalamus, the corpus callosum, the internal capsule and the putamen, and for an infusion flow rate of 4 µl/min (Fig. 4) [31]. Results showed that injections via the gray matter (thalamus injection) yielded to a Vd of 80% in the caudate nucleus because of the relatively short distance between the injection site and the target and because of an isotropic uniform structure. On the contrary, injections via white matter tracts (the corpus callosum and the internal capsule) impregnated the caudate at 72 and 60%, respectively. When injecting into anisotropic media, the infusate travels long distances due to the higher permeability along white matter tracts, resulting in more infusate loss and consequently, less quantity available for diffusion into the target. Although white matter targets were required to achieve high Vd levels, it was not recommended to place the catheter in an anisotropic structure if the target was not located at the cannula place. Finally, although it was qualified as a gray-matter structure, putamen injections were worst because the studied target received only 10% of the initial drug. This can be explained by the larger distance between the two structures and the presence of white fibers between them.

2.2.3. Rate of infusion – catheter size

The pressure gradient, which generates the convective movement, is equal to the difference between the skull pressure and the injection pressure. The flow of injection is thus a critical parameter to create convection, and it is known that it is related to the resistance of the considered tissues (gray and white matter). Finding an optimal infusion rate for CED has been elusive because it is often limited by the development of backflow along the cannula track. In most cases, the optimal infusion rate is that which allows the delivery of the therapeutic volume over the least amount of time without any associated reflux. This optimum is also dependent on the cannula size used. In general, the higher the infusion rate and catheter diameter used, the greater the reflux induced.

To obtain effective convection in rodent models, the injection flow must be in the range of 0.5–5 µl/min [14,39]. Indeed, weaker flows limit the extent of the distribution volume, whereas too high flow facilitates backflow. In addition, the use of superior flow levels is not recommended as the generated hydrostatic pressure can damage the tissues [45]. Consequently, the use of a 0.5 µl/min rate of infusion is often described to carry out effective CED in rodents [19,27,42,46]. Kroll et al. underline that the infusion rate has to be adjusted according to the model used [47]. When they attempted to establish a rat infusion rate of 4 µl/min as reported by Bobo et al. in their study in cats [14], leakage up and out of the catheter occurred. Whereas injections took place in white matter
for cats, the organisation of the fibers in rat gray matter was characterized by increased resistance, inducing backflow.

In addition, the larger the catheter diameter, the greater the tissue resistivity and reflux induction. Chen et al. showed that leakback associated with the smallest cannula (32 gauge) was significantly smaller than that associated with the two larger cannulae: 28 and 22 gauge [48]. An increase in cannula diameter facilitates the formation of a low-resistance pathway that follows the surface contours of the cannula. Bauman et al. demonstrated that, at a fixed flow rate, the backflow distance varies as the four-fifths time of the outer diameter (OD) of the catheter [49].

Moreover, volumetric inflow rates associated with catheter diameter must be carefully chosen to avoid loss or bad distribution associated with backflows that reach the outer boundaries of the target structure. In some studies using low infusion rates, the infusate is almost entirely contained in the target (striatum) whereas at rates ten times higher, there is significantly more infusate outside the target [48,50]. For example, with a 32-gauge catheter, confinement of the infusate in the rat caudate (radius of 0.22 cm) requires a flow rate not greater than 0.5 μl/min. In fact, at this infusion rate, the backflow distance is equivalent to 0.2 cm whereas it is near 0.8 cm for an infusion rate of 5 μl/min. Moreover, for a 22-gauge catheter, the backflow distance increases from 0.5 to 2 cm for flow rates equivalent to 0.5 μl/min and 5 μl/min, respectively [50]. The significance of these observations is that the selection of a too high flow rate into a gray matter target will lead to diminished delivery and internal leakage to nearby white matter regions and perhaps to external leakage as well.

Other studies performed in human brains showed that for a fixed infusate flow rate, the smaller the catheter used, the greater the volume of distribution, due to higher velocities at the tip [31]. Recent clinical trials using high infusion flow rates (3.3 μl/min) and large catheters (1.4 and 2.5 mm OD) described MRI signal changes in the catheter track after infusion in patients due to reflux [51]. We can say, however, that as the volume of brain structure is larger in humans, there is a much higher flexibility concerning the infusion

![Diagram of brain sections](image-url)

Fig. 4. Coronal brain section identifying the location of gray (Gm) and white matter (Wm) in humans (A) and in rats (B).
rates employed. Recently, experimentators have increased the infusion rate to 5 μl/min without any reflux thanks to the use of an innovative cannula design [45].

2.2.4. Catheter design

The use of a reflux-free cannula in order to enhance the infusion rate of therapeutic agents by CED has been described, thus reducing the duration of treatment and, by the way, the exposure of patients to high risk of infection or side effects [45]. The design used in this study consisted of a 27-gauge cannula needle with glued-in fused silica tubing attached to the CED infusion system. This catheter allowed the delivery of infusate (0.4% Trypan blue solution) at a flow rate of 50 μl/min without any reflux in agarose gel, and rodent and non-human primate brains. But, if there was no leakage, high levels of induced tissue damage appeared in animal models when infusion rates were above 10 μl/min.

Multiple-hole catheters are cannulae with 5–6 holes of a few millimeters (outer diameter) on each side, separated at short distances. Studies have shown that the use of this type of catheters leads to high volumes of distribution due to the distributed arrangement of outlet ports [31]. On the contrary, by studying the arrangement of outlet ports [31]. On the contrary, by studying the ters leads to high volumes of distribution due to the distributed distances. Studies have shown that the use of this type of cathe-

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2.2.5. Brain extracellular matrix dilatation

The brain’s extracellular matrix (ECM) is the conduit through which drugs and drug delivery nanovectors must diffuse after crossing the BBB or after direct brain administration by CED. If the surface of this brain ECM can be enhanced, the volume of distribution may be increased. Neeves et al. speculated that, like osmotic and hydraulic dilatation, enzymatic degradation of the brain ECM could enhance the distribution of nanometric objects [54]. To investigate the hydraulic dilatation hypothesis, they injected PBS prior to nanoparticle infusion (30 min before) and they showed that the distribution volume was more than twice as large as the distribution volume of the control group treated by NP in PBS. However, it was difficult to compare these two results as the total infused volume was twice as high for the rats which underwent hydraulic dilatation. Furthermore, degradation of hyaluronic acid by the co-administration of hyaluronidase at 20,000 U/ml for 30 min before the injection of NP resulted in a 58% increase in the distribution volume of nanoparticles. These results had to be taken with care as the consequences of a hyaluronic depletion are not clearly known and especially in a contact with brain tumor. These results showed that both enzymatic treatment and dilatation of the extracellular space could significantly enhance the transport of nano-objects.

2.2.6. Heart rate enhancement

In order to increase the distribution volume, it should be possible to enhance brain fluid circulation by enhancing the level of this circulation. Hadaczek et al. hypothesized that infusion distrib-

2.2.7. Volume and nature of the infusate

Vd determination in CED is a complex process which includes the intervention of many parameters. However, when infusions are performed into regions of interest with adequate materials, the Vd is characterized by a direct linear relationship with Vi which represents the volume infused. This information has been checked in different species such as cats [14], rats [56], pigs [40], dogs [57], non-human primates [42,58], and humans [59,60]. The relationship between Vi and Vd is linear, but the ratio Vd/Vi is dependent on structural properties of the tissue on the one hand, and characteristics of the infusate on the other hand. Heavy molecules like trophic factors or proteins diffuse more slowly than small mole-

sulfate proteoglycan was very high and can explain their lower diffusion levels as these receptors are found in all neurons. This conclusion was correlated to the study of Nguyen et al. who used heparin-like co-infusate to saturate receptors and in this way, to enhance the distribution of AAV2 particles [27].

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and targeting possibility. The nanocarrier family includes mainly polymeric or lipid-based carriers such as liposomes, nanoparticles (including nanospheres and nanocapsules), micelles, and dendrimers (Fig. 5). With drug delivery, the pharmacokinetic properties of the drug will no longer depend on the properties of the active molecule but on the physicochemical properties of the carrier. Thus, nanoencapsulation offers many advantages such as the protection of sensitive active molecules against in vivo degradation, the reduction of toxic side effects which can occur when drugs are administered in solution, better drug pharmacokinetic behavior, and an increase in patient comfort. Thanks to the possibility of grafting specific ligands to their surface, nanocarriers can recognize specific targets [66]. Nanoparticles can also bypass multidrug resistance (MDR) mechanisms by inhibiting efflux pumps such as P-glycoprotein (P-gp), and optimizing the bioavailability of anticancer agents [67–69].

After CED injection, nanocarriers have to diffuse themselves in extracellular brain conduits. Consequently, the size of these nanocarriers appears to be a critical parameter for the delivery of drugs into the brain. Nanocarriers that have already been injected by CED are liposomes [43], nanoparticles [29,46], dendrimers [30] and polymeric micelles [70]. Liposomes are artificial phospholipid vesicles varying in size from 50 to 1000 nm and even more, which can be loaded with small therapeutic agents including drugs and genes, and have been considered as promising drug carriers since over three decades. Hydrophilic drugs can be readily entrapped within the aqueous interior of the vesicles, and neutral and hydrophobic molecules can be carried within the hydrophobic bilayers of the vesicles. Liposomes can provide stable encapsulation and the delivery of a number of potent anticancer drugs [71,72].

Nanoparticles are defined as solid colloidal particles ranging in size from 10 to 1000 nm. They consist of macromolecular materials or lipids in which the active principle (drug or biologically-active material) is dissolved, entrapped, encapsulated and/or to which the active principle is adsorbed or attached [73,74]. They are constituted in general from biodegradable and non-biodegradable

### Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Description</th>
<th>Vd</th>
<th>Backflow</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain geography</td>
<td>White matter</td>
<td>High conductivity</td>
<td>+++</td>
<td>[14,41,42]</td>
</tr>
<tr>
<td></td>
<td>Gray matter</td>
<td>Low conductivity</td>
<td>+</td>
<td>[47]</td>
</tr>
<tr>
<td></td>
<td>Tumor tissue</td>
<td>Modified conductivity</td>
<td>+</td>
<td>[15,43,104]</td>
</tr>
<tr>
<td>Catheter placement</td>
<td>Location</td>
<td>Bad insertion</td>
<td>+</td>
<td>[17,44]</td>
</tr>
<tr>
<td></td>
<td>Distance to target</td>
<td>Large distance</td>
<td>+</td>
<td>[31]</td>
</tr>
<tr>
<td></td>
<td>Distance to target</td>
<td>Short distance</td>
<td>+</td>
<td>[31]</td>
</tr>
<tr>
<td>Catheter size</td>
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<td>&lt;28 gauge</td>
<td>+</td>
<td>[47,48]</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>≥32 gauge</td>
<td>+</td>
<td>[47]</td>
</tr>
<tr>
<td>Infusion rate</td>
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<td>+</td>
<td>[50,51]</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>≤0.5 μl/min (rodents)</td>
<td>+</td>
<td>[19,27,46]</td>
</tr>
<tr>
<td>Catheter design</td>
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<td>Glued-in fused silica tubing</td>
<td>+++</td>
<td>[45]</td>
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<td></td>
<td>Multiple hole</td>
<td>Irregularly shaped Vd</td>
<td>+</td>
<td>[31,49]</td>
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<td></td>
<td>Single end port</td>
<td>Spherical distribution</td>
<td>+++</td>
<td>[49]</td>
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<td></td>
<td>Bone wax fixed</td>
<td>Increased pressure</td>
<td>++</td>
<td>[53]</td>
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<td></td>
<td>Primed cannula</td>
<td>To prevent air bubbles</td>
<td>++</td>
<td>[52]</td>
</tr>
<tr>
<td>Infusion volume</td>
<td>Increased V̄</td>
<td>Linear relationship Vd/V̇</td>
<td>+++</td>
<td>[14,61]</td>
</tr>
</tbody>
</table>

Vd: distribution volume, V̇: infusate volume.

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**Fig. 5.** Schematic structure of nanoparticles including nanospheres (A) and nanocapsules (B), dendrimers (C), liposomes (D) and micelles (E) for drug delivery.
polymers, or from lipids. They can be classified into two groups according to their inner structure, namely nanospheres and nanoparticles [75,76]. Dendrimers are spherical, highly-branched polymers with a hierarchical three-dimensional structure [77]. They look treelike in their molecular architecture since they are built from repetitive monomers with branching point units that are radially connected around a template core [78]. Polymeric micelles are supramolecular assemblies of amphiphilic block copolymers with a core–shell structure which maintains physical properties characteristic of conventional micelles, but with enhanced thermodynamic stability [79]. All these nanocarriers, when administered by CED, have a double application: firstly, they can be used as a tracer to monitor the infusion; secondly, they can be loaded with antineoplastic agents for an anticancer therapy approach.

### 3.1. Nanocarrier labelling

Nanocarriers can be labelled by incorporating a marker in the liposomal/nanoparticle membrane and/or can be loaded by encapsulation of a marker within their interior part (Fig. 6). Such applications involve the use of histological [80] or fluorescent markers [81,82], radiotracers [83–86], and MRI contrast agents [87,88]. The ideal conditions are obtained when nanocarriers can be labelled and loaded by two kinds of markers, thus excluding the possibility of a distribution linked to the marker released from the carrier. Moreover, nanocarrier labelling, especially radiolabelling, can give information about brain residence time and in situ degradation [46,84]. Lastly, MRI contrasts agents are the main interesting markers because they allow real-time imaging acquisitions. These molecules are able to modify the longitudinal (T1) and transverse (T2) relaxation times. The ability of a contrast agent to shorten T1 and T2 is defined as the relaxivity, $r_1$ or $r_2$, respectively. In general, there are two classes of MR contrast agents. On the one hand, there are agents that have a low $r_2/r_1$ ratio and therefore generate positive contrast in T1-weighted images. These positive contrast agents usually are paramagnetic complexes of Gd$^{3+}$ or Mn$^{2+}$ ions [89,90]. On the other hand, there are super-paramagnetic contrast agents with a high $r_2/r_1$ ratio, which cause dark spots in T2- and T2*-weighted images and are therefore referred to as negative contrast agents. These contrast agents are usually based on iron oxide particles [91,92]. The two kinds of MR contrast agents were encapsulated in nanocarriers for CED injection. As liposomes can be loaded with hydrophilic molecules, it was possible to formulate liposomes containing paramagnetic agent such as Gdteridol, a chelated gadolinium (Gd). Saito et al. established a method for using MRI to monitor the CED of liposomes in the brain in real time [93]. They concluded that, although histological methods are much more sensitive, MRI monitoring provides distribution patterns with precise information and in real time. With this technique, they were able to see the difference between the invasive properties of two tumor models in rats such as the C6 and the 9L-2 glioma models. In a word, MRI appears to be a real-time imaging technique to track the brain delivery of liposomes [94].

Perlstein et al. evaluated the distribution of maghemite nanoparticles (Fe$_3$O$_4$–MNP) of approximately 80 nm average diameter, prepared by controlled nucleation and growth of thin maghemite films onto appropriate nuclei [95]. These superparamagnetic particles were injected by CED in normal rat brain with different viscosities and evaluated regardless of their diffusion properties [29]. The results showed a very good correlation between MRI and fluorescent acquisitions after loading the Fe$_3$O$_4$–MNP with rhodamine, establishing the use of MRI for reliable maghemite nanoparticle imaging. Nevertheless, superparamagnetic iron particles are characterized by a very high sensitivity to MR imaging [96]. Thus, iron signals sometimes give excessive results, especially when compared to histological findings. After the CED administration of iron particles in rat brain, the Vd measured by MRI was overestimated by a factor of 0.38 compared to histological methods [47].

### 3.2. Nanocarrier physicochemical properties

In this chapter, we will focus on the parameters influencing a nanocarrier’s aptitude to diffuse in brain parenchyma, depending on their size, charge, composition, surface properties, and physicochemical characteristics. All these data are resumed in Table 2.

#### 3.2.1. Size

Many studies have focused on the optimum in size for nanocarriers used in CED. The conclusions are quite unanimous since the distribution volume of nanocarriers in rat striatum is inversely proportional to the size of the particle. Mackay et al. worked on the physicochemical properties of liposomes in order to optimize post-CED diffusion [84]. They concluded that ideal liposomes for CED should be less than 100 nm in diameter, because above this size, liposomes are retained near the site of injection and are characterized by restricted mobility. Studies on brain extracellular space ( ECS) gave more precise informations: the ECS has been estimated at between 35 and 64 nm in diameter in normal rat brain [97] which means that many vectors beyond 100 nm will be too large to transit normal neocortical extracellular space. The size of some polystyrene nanospheres administered by CED was also evaluated in rats in order to mimic the behavior of viral vectors [98]. The conclusions were that the Vd of these nanocarriers in rat striatum were about 9 mm$^3$ for 20 nm nanospheres and about 1 mm$^3$ for 100 and 200 nm particles. Nevertheless, when the nanospheres were covered by albumin, the effect of size was reduced. Indeed, albumin coating can mask the hydrophobic structures of the polystyrene nanospheres, reducing the risk of the eventual aggregation and binding to proteins in the extracellular space.

#### 3.2.2. Surface properties

Surface properties have a considerable impact on the diffusivity of colloidal vectors especially because of the presence of a steric coating. Polyethylene glycol (PEG) and dextran coating significantly increase the distribution of liposomes and nanoparticles delivered by CED [29,84]. Biocompatible polymers such as dextran and PEG are known to extend the systemic circulation of such nanocarriers because they significantly reduce interactions with proteins [99,100]. Following CED infusions, these same excipients may reduce the binding of nanocarriers to brain cells, allowing
a greater diffusion compared to those without any coating. Surface charge has also been studied concerning the diffusivity of liposomes in the rat brain. Mackay et al. observed that when liposomes were charged with modest amounts of positive charge (10% per Mole lipid), the distribution of such liposomes was significantly decreased compared to neutral nanocarriers ($p < 0.0005$) [84]. Cationic liposomes were found adjacent to the needle tract because of non-specific binding to negatively-charged structures in the brain parenchyma [101]. The diffusivity of cationic liposomes is a challenge as they are used as vectors for gene delivery [102]. However, the pegylation of these carriers enhanced the distribution volume reducing tissue affinity as demonstrated above [103]. On the contrary, negative charges have no effect on the diffusivity of such liposomes. Based on these findings, a neutral or negative surface charge is required to obtain a good diffusion [84].

### 3.2.3. Osmolarity

The use of mannitol has been described to increase the convective effects of liposomes, especially a few hours after infusion. The CED of 40 nm-liposomes co-infused with 25% mannitol produced an enhancement of the distribution from 52.5 ± 2.1 to 78.5 ± 5.5 units immediately after CED and was even more pronounced after 48 h (34.8 ± 7.5 compared to 13.5 ± 3.2 units) [104]. This phenomenon was explained by the hyperosmotic power of mannitol which may increase the size of channels of the interstitial space through which liposomes could transit. Similarly, Neeves et al. co-infused BSA-coated polystyrene nanoparticles with 25% mannitol (Osmolarity = 1568 ± 12 mOsmol/kg) in normal rat brain and showed that the distribution was enhanced by about 50% [54].

On the contrary, Chen et al. studied the influence of BSA solution osmolarity and revealed that there were no dramatic effects on the Vd when the BSA osmolarities varied between 145 and 450 mOsmol/kg [98]. They, then, injected nanospheres coated with BSA and studied the Vd in rat striatum. They concluded that the distribution of nanospheres coated with BSA was not affected by osmolarity. But, they considered that the transport of such a small molecule as BSA (7.2 nm) was comparable to nanocarriers of about 20–200 nm in size. The diffusivity of nanoparticles in porous media was reduced when compared to that of small molecules in solution because of a combination of hydrodynamic and steric factors [105,106].

### 3.2.4. Viscosity

By increasing the viscosity of the infusate, Mardon et al. demonstrated that CED efficacy could be enhanced [107]. In fact, a linear correlation was found between Vd and viscosity. It is easy to increase the viscosity of a nanocarrier formulation by dissolving sugar or polymers in the aqueous external phase of such a suspension. Perelstein et al. increased the viscosity of their nanoparticle suspension by incorporating PEG (3–6%) or sucrose and the results were characterized by an improved distribution capability [29]. High viscosity of the infusate may also reduce backflow, thus increasing the possibility of efficient convection. Moreover, the efficient formation and extent of convection obtained by using high-viscosity infusates enable the coverage of larger volumes of distribution in less time, thus reducing the time of infusion and consequently the appearance of related side effects [108]. After the CED injection of two formulations of carboxylate (4 mg/ml) and carboxylate + sucrose (4 mg/ml + 12%), Mardon et al. showed that not only the Vd was enhanced but also the cytotoxic treatment effects measured by MRI (performed on Days 1 and 4) [107]. This significant correlation suggests that, by increasing the infusate viscosity, it is possible to significantly increase the efficiency of CED, and consequently improve the efficacy of treatment.

#### 3.2.5. Concentration

Chen et al. showed that the concentration of the infusate had no impact on the calculated Vd, nor on the global distribution of the infusate in brains [48]. Indeed, when the 14C-BSA concentration increased from 25 to 50 and 100%, the corresponding Vd were about 20.6 ± 1.8, 21.5 ± 2.6 and 19.6 ± 2.7 mm³, which means that the differences in concentration did not alter the distribution pattern. They explained that the administration of a pharmacological agent by convection is based on the transport of a material through the interstitial space which is not dependent on concentration gradients as in diffusion methods. But the reality is more complex and seems to be linked to the nature of the materials infused. When infusion is carried out with monocrystalline iron oxide nanocompounds (MIONs), Kroll et al. concluded that concentration was a principal parameter [47]. Evaluating which parameters between dosage, volume and infusion time, may have the greatest influence on increasing the Vd, they evidenced a major impact of the dose effect. By increasing the iron dose contained in monocrystalline iron oxide nanocompounds (MIONs) from 5.3 to 26.5 μg, the MRI calculated Vd increased by 4.9- and 2.5-fold for infusion rates of 0.2 and 1.2 μl/min, respectively. The overall effect of dosage, at these two different rates, was significant ($p < 0.001$). It is especially interesting to note that the Vd associated with either dose was greatest with the lowest flow rate tested because of the occurrence of backflow for the higher rate. Even if superparamagnetic iron particles are known to overestimate the signal, histological sections confirmed the same Vd ratio for the different formulations tested. With the same idea in mind, Mackay et al. demonstrated that when the liposome concentration was increased, the distribution volumes also increased. They postulated that the liposome-engulfing cells could be responsible for reducing the penetration of liposomes into the brain. This process would require particle adsorption, and the excess of liposomes was used to block the binding sites reducing the adsorption of the nanocarriers left along the conduction pathway [84].
3.2.6. Brain affinity

The use of co-infusates (e.g. heparin, basic fibroblast growth factor or mannitol) has been widely described as reducing the affinity of infusates to the brain environment. Hamilton et al. demonstrated the influence of receptor binding on the distribution of trophic factors in the CNS [109]. They showed that heparin co-infusion significantly enhanced the Vd of GDNF and GDNF-homologous trophic factors, probably by binding and blocking heparin-binding sites in the extracellular matrix. The same observations were made to enhance the distribution of viral particles such as adeno-associated virus type 2 (AAV2) particles [27]. Working on the same vectors, Hadaczek et al. indicate that the simultaneous injection of basic fibroblast growth factor (bFGF) with AAV2-thymidine kinase (AAV2-TK) can greatly enhance the volume of transcuded tissue, probably by the way of a competitive block of AAV2-binding sites within the striatum [110]. Similarly, heparan sulfate proteoglycans (HSPGs) have been identified as primary viral receptors [111] and are known to be abundantly present on neurons [112,113]. The use of mannitol was described as blocking the adeno-associated virus type 2 (rAAV) binding to HSPG thus facilitating the spread of the virus [114]. For a nanosized structure, a compromise had to be made between weak interactions with brain ECM and cellular internalization after 12 h. On the other hand, interaction with brain ECM has to be controlled for optimal distribution, but on the other hand, the carrier loaded with its anticancer agent has to play its ‘toxic’ rule to eradicate the tumor. Most of the time, anticancer agents have to penetrate the cells to be active as their targets are sub-cellular entities.

3.2.7. Pharmacokinetic behavior

Modification of drug pharmacokinetic behavior is one of the many advantages linked to nanocapsulation strategy [115]. For example, Saito et al. compared the distribution of doxorubicin hydrochloride, which is a small, hydrophilic molecule, infused by CED, with a pegylated liposomal formulation of doxorubicin (Doxil®) [103]. The results showed that the drug alone had poor tissue distribution and did not cover the entire tumor mass, whereas liposomal formulations did. Due to the affinity of free doxorubicin for cellular components, the accumulation of free doxorubicin was found in cellular nuclei whereas most of the Doxil® was found in the intercellular spaces.

However, elimination routes for the nanocarriers infused by CED may vary substantially between formulations and need to be understood. Each nanocarrier infused by CED can be characterized by a brain half-life which is defined as the time when half the quantity has disappeared from the brain. For example, Mackay et al. described a brain half-life of about 9.9 h for neutral pegylated liposomes and 15 min for positively-charged liposomes [84]. We have previously shown that the elimination of lipid nanocapsules (LNC) loaded with a hydrophobic complex of rhumen–188 (188Re-SSS) was significantly lower when compared to a hydrophilic solution of rhumen–188 perrhenate [46]. The CED infusion of Rhumen–188 perrhenate solution revealed that more than 80% was eliminated in 12 h and about 94% after 72 h post-CED. 188Re perrhenate brain half-life (brain t1/2) was equivalent to 7 h, and the solution was mainly excreted by the kidneys as 99.4% of the total excreted radioactivity was recovered in urine. On the contrary, only 10% of rhumen–188 encapsulated in LNC was detected in urine and feces 72 h post-CED infusion. 188Re-SSS LNC were found to be a sustained residency system which constitutes a major advantage (1% elimination after 12 h). The lipophilic nature of LNC and their intracellular glioma location [116] leads to a long radionuclide brain retention time allowing improved tumor irradiation. Maghemite nanoparticles (Fe3O4–MNP) were also characterized by a high time residency (t1/2 brain = 10 d), with about 10–20% remaining at Day 40 and no toxicity being observed up to 120 days after CED [29]. This long time residency was also correlated with an intracellular co-localization or surface adsorption of the Fe3O4–MNP as detected after Prussian blue staining. If the infusion time surpasses the nanoparticle half-life, it is impossible to deliver the particle for large volumes without blocking the source of elimination. On the contrary, if it is possible to increase the brain half-life, particles can be infused for longer periods and achieve greater volumes of distribution.

4. Survival studies and clinical trials

In the context of CED, nanocarriers can be used as vectors to track infusion and/or as vectors to treat solid tumors. As the results are different between species because of difference in brain structure, we separated the results obtained from rodent brains and larger brain models including studies on non-human primate brains and dogs. To conclude, a few comments have been made for clinical trials involving the use of nanocarriers. The main preclinical and clinical studies involving the use of nanocarriers infused by CED are summarized in Table 3.

4.1. Rodent models

Pegylated liposomal doxorubicin (Doxil®, Alza Pharmaceuticals, Inc., Mountain View, CA; Caelyx®, Schering-Plough, Inc., Kenilworth, NJ) and liposomal daunorubicin (DaunoXome®, Gilead, Inc., Foster City, CA) are approved as liposomal formulations for clinical use [117–119]. Saito et al. proved the concept feasibility of a co-infusion of Doxil® and liposomes loaded with gadolinium to provide direct evidence of the drug Vd in tissue during CED [43]. The conclusions were that CED effectively distributed Doxil® liposomes in the tumor and the surrounding normal brain tissue that contained invasive tumor cells. The CED of Doxil® into rodent brain tumors demonstrated that doxorubicin is present in the tissue several weeks after a single administration [120].

In terms of pure cancer therapy, a novel nanoliposomal formulation has been developed and loaded with a campthotecin derivative, Irinotecan or CPT-11, in order to use it in rodent brain tumor models [121]. Following CED in rat brains, the tissue retention of nanoliposomal CPT-11 (nLs-CPT-11) was greatly prolonged as compared to free drugs, with >20% of the injected dose remaining at 12 days for all doses. Brain tissue residence was dose-dependent and increased as the dose increased. At equivalent doses, brain t1/2 was 22-fold higher for nanoliposomal CPT-11 than for free CPT-11, without signs of toxicity, increasing the tolerance of the molecule, more than 4-fold. The median survival time of rats bearing 9L-2 glioma was largely increased for the group treated by the highest dose tested (1.6 mg/rat) with 62.5% of the rats treated being long-term survivors (>100 days) [28]. Although no great difference was seen in low-dose free CPT-11 versus low-dose nLs-CPT-11, the fact that high-dose nLs-CPT-11 could be used in the absence of limiting brain toxicity confirmed the possibility of the direct clinical application of this technology for the treatment of brain tumors.

The same kinds of results were observed with nanoliposomal Topotecan (nLs-TPT). Topotecan is a water-soluble camptothecin derivative that inhibits the topoisomerase I leading to DNA damage in tumors. The analysis of brain tissue revealed a dramatic improvement in brain retention of nLs-TPT as compared to free drugs as the brain half-lives were about 1.5 and 0.1 days, respectively. The study showed that the CED of nLs-TPT resulted in the perivascular accumulation and consequent disruption of tumor vessels suggesting a possible antiangiogenic mechanism of liposomes loaded with a chemotherapeutic cargo. The CED of nLs-TPT inhibited growth or completely eradicated orthotopic U87MG or
Liposome inhibitors and a significant improvement in survival.

The results showed a synergistic effect between the two topoisomerase I inhibitors and a significant improvement in survival xenograft studies: this may be due to the reduced growth of U251MG cells compared to U87MG and nLs-CPT-11 were also mixed and evaluated with respect of toxicity, tissue half-life, and efficacy in vivo model tumor cell lines.

The synergy between the two agents was only observed in one of the two cell lines tested (U251MG), in in vitro cell cycle profile as well as in vivo survival xenografts studies.

The results showed a synergistic effect between the two topoisomerase I inhibitors and a significant improvement in survival time compared to the control groups and to the groups treated with both drugs separately. These results were all the more significant as the liposome treatment took place 10 days after cell inoculation, which means that the tumor was an advanced tumor with a well-established vasculature.

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Doxil® and nLs-CPT-11 were also mixed and evaluated in vitro and in vivo with respect of toxicity, tissue half-life, and efficacy in U87MG and U251MG xenografts to learn about the synergy of action of these two molecules [124]. Although the dose of Doxil® used was 400-fold lower than that of nLs-CPT-11, their brain half-lives were similar and equivalent to 16.7 and 10.9 days, respectively.

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higher sensitivity to the doxorubicin and irinotecan combination. Even so, a better liposome distribution was observed in U87MG xenografts when compared to U251MG tumors characterized by the presence of large necrotic areas. This means that the growth of the cell lines used for implanted xenografts was a principal factor for in vivo survival results.

Dendrimers are synthetic polymers with a well-defined globular structure. They are composed of a core molecule, repeated units that have three or more functionalities, and reactive surface groups. A fifth generation (G5) polyamidoamine dendrimer containing methotrexate (MTX), a folate antimetabolite-like anticancer agent covalently linked to cetuximab, a monoclonal antibody was prepared and administered by CED in rats with F98 gliomas [30]. Cetuximab (Erbitux® or IMC-C225) was able to bind the epidermal growth factor receptor (EGFR) and its mutant isoform (EGFRvIII), frequently overexpressed in malignant gliomas [125–127]. The amount of the so-called C225-G5-MTX dendrimer after CED was more than 5-fold higher in EGFR-positive gliomas compared to receptor negative tumors, which means that there was direct interaction between the vector and the glioma cells. Unfortunately, there was no impact on the median survival time as no significant difference between the control group, the group treated by the C225-G5-MTX dendrimer, and the group treated by MTX alone was observed. This could be due to a loss of activity of MTX after the linkage of this antifolate molecule to the dendrimer structure, altering the interaction with dihydrofolate reductase which is essential for a biological activity. Moreover, no investigation was carried out concerning the diffusion of this carrier C225-G5-MTX dendrimer in the rat brain.

Dendrimers have also been used for boron neutron capture therapy (BNCT) [128,129]. Briefly, BNCT is a binary radiation therapy modality that brings together two components that, when therapy (BNCT) [128,129]. Briefly, BNCT is a binary radiation therapy modality that brings together two components that, when combined, provide a highly localized lethal dose of radiation to malignant tissue. BNCT is particularly advantageous for the treatment of brain tumors due to the high concentration of boron-containing compounds in the brain and the low concentration of these compounds in other organs. The boron isotope 10B is used in BNCT, which is selectively concentrated in certain tumor cells that overexpress the epidermal growth factor receptor (EGFR) and its mutant isoform (EGFRvIII), frequently overexpressed in malignant gliomas [125–127]. This selective uptake of boron into the tumor results in a high concentration of boron at the site of the tumor, allowing for a high dose of radiation to be delivered to the tumor while minimizing damage to surrounding healthy tissue.

In order to work in conditions very close to human ones, and as a strategy to target different structures of the brain, Saito et al. aimed at targeting corona radiata, putamen and brainstem with a maximum of 100 µl-liposome infusion; they described robust and clearly-defined distribution of liposomal Gd in each infusion site [93]. Then, they injected a fixed and considerable volume of liposomes (700 µl) into each of these three regions to investigate the regional difference in brain structure. The results of all three infusion sites showed a linear correlation between Vi and Vd with a maximum distribution observed in the brainstem (Vi/Vi = 2.3) and a minimum for the putamen (Vi/Vi = 1) [133]. Lonser et al. described a Vd/Vi ratio of up to 8.7 in primate brainstem with an infusion of albumin-bound Gd, showing that not only the brain region had to be considered, but also the physico-chemical properties of the infusate [42]. The limited size of primate putamen seems to restrict Vi and was characterized by a leakage of liposomes out of this structure. This leakage was correlated with the perivascular transport of liposomes throughout CNS arteries [134]. These findings suggest that every therapeutic agent infused into putamen should be closely monitored for distribution, since the possibility of side effects increases greatly with leakage out of the infusion site. Nevertheless, a principal advantage of CED is that liposomal distribution stops immediately once the CED pumps are turned off.

In order to mimic a real intervention for human brain-tumor patients, three consecutive infusions of liposomes with an increased volume of infusate were performed in the same region in a primate brain. No change in distribution was noted after repeated infusions and the linear relationship between Vi and Vd was also maintained in the brainstem and in the corona radiata. After histological examinations of the right and left hemispheres, no modifications were noted in both parts of the brain, suggesting that multiple injections appear to be a safe and conceivable treatment approach. In order to work in conditions very close to human ones, and as only a few data can be collected concerning the distribution in tumor models, the next step will be the administration of such nanocarriers in large models bearing tumors. Dogs are able to
generate spontaneous brain tumors with an incidence near to that observed in humans. Moreover, canine tumors are on the scale of human tumor patients with biological, histological and molecular characteristics very similar to those reported in humans [135–138].

For the first time, Dickinson et al. investigated the infusion of liposome by CED in a canine model but in healthy brains [57]. They injected a mixture of liposomes loaded with Gd and with CPT-11 as a potential treatment strategy. The CED resulted in robust Vd in both gray and white matter, with minimal adverse effects. Leakage was only observed in one of the 11 infusions performed and was mainly due to poor catheter placement. No adverse clinical effects were associated with leaving the infusion cannulae in situ, and second infusions were successful in all cases. In brief, this study confirmed the results observed in rodents and primate studies on normal brains. The next step will be the administration of nanocarriers in spontaneous canine tumors first, and in human glioma very shortly.

4.3. Clinical trials

The clinical trials randomized patients who had failed conventional therapy (surgery, radiotherapy and/or chemotherapy) [139]. The first therapeutic agent infused via CED for malignant glioma was diphtheria toxin conjugated to transferring Tf-CRM107 [140]. This active agent belongs to the targeted toxin family which is mostly composed of recombinant polypeptides designed with two segments that represent a new class of agents with a high specificity for tumor cells [141–143]. Other clinical trials using CED reported in literature are trials infusing anticancer drugs like...
paclitaxel [17], or radioimmunotherapy drugs like Cotara®, a 131I-labelled chimeric monoclonal antibody [21].

Among the clinical trials described in the literature using CED, only a few studies described the use of drug-loaded nanocarriers. The concept of gene therapy has been investigated to fight against malignant glioma and has been the object of encapsulation in nanocarriers such as liposomes. Ren et al. studied the anticancer efficacy of a genetically-modified replication-disabled Semliki forest virus vector (SFV) carrying the human interleukin 12 (IL12) gene, encapsulated in cationic liposomes (LSVF-IL12) [144]. Virus encapsulation has several advantages such as reduced recognition of the virus by the immune system, virus protection from an in vivo inactivation process and a prolonged in situ residency time. Unfortunately, this study gave no therapeutic results and settled for providing the description of the phase I/II clinical protocol.

Other studies have investigated the encapsulation of a retrovirally mediated HSV-1-tk gene transfer, which sensitizes tumor cells for ganciclovir (GCV) in liposomal structures [145,146]. In this concept, the use of synthetic nanocarriers has been investigated to avoid the use of viral vectors because of easier preparation, a lack of immunogenicity and higher stability over time. In fact, Voges et al. presented the results of a prospective phase I/II clinical study using CED of an HSV-1-tk gene-bearing cationic liposomal vector (LIPO-HSV-1-tk) and systemic GCV for the treatment of GBM [147]. The results showed that the treatment was tolerated with minor side effects indicating the safety and feasibility of this technique. Within the 8 patients treated, two of them had a 50% reduction of tumor volume, whereas for the others, only a focal effect was noted. Nevertheless, the main critical aspect of this study was that the monitoring of the CED injection by MRI was performed after the injection of Gd-DTPA (550 Da, no charge) and not with the liposomes encapsulating Gd (180 ± 20 nm, negative charge) given that the distribution volume is known to be strictly dependent on the physicochemical properties of the nanocarrier.

5. Conclusion

Local delivery of agents to brain tumors by Convection-Enhanced Delivery offers the advantage of better drug distribution compared to other strategies only governed by diffusion. Because this technique was also characterized by the appearance of side effects caused by backflow along the catheter and drug leakage in non-desired regions, the encapsulation of active molecules within the concept of CED has been investigated to overcome this problem. The encapsulation of such a drug, a toxin, or a gene is under investigation on experimental models rather than in clinical trials, but seems to be very promising for the treatment of brain malignancies. In the context of solid brain tumors, the nanocarriers have to be characterized by a high drug loading level to eradicate the tumor and to be labelled with a contrast agent in order to realize real-time imaging. In terms of pure structure, the ideal nanocarrier would be about 20–50 nm in size, with a global neutral or negative charge, and shielded by a steric coating made of PEG or dextran (Fig. 7A). The final infused suspension would be viscous, hyperosmolar, with the eventual presence of co-infused to saturate the binding sites along the nanocarrier route (Fig. 7B). It should be infused at high concentrations especially for carriers that have to target the intracellular compartment. The elimination route has to be controlled in order to prevent the rapid elimination by blood capillaries in a brain extracellular matrix (Fig. 7C). In a word, the objective for using CED for drug delivery nanomaterials is twofold: Firstly, thanks to specific structural properties, the nanocarrier has to diffuse into the brain parenchyma in order to obtain an optimal volume of distribution to cover tumor mass and infiltrating cells. Secondly, the nanocarrier has to be internalized by cancer cells in order to exert its cytotoxicity, mediated by anti-cancer agents.

Acknowledgements

This work was supported by a ‘Région des Pays de la Loire’ grant and by the ‘Cancéropolis Grand Ouest’.

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