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REVIEW

Nanoparticle Technology for Drug Delivery Across the Blood-Brain Barrier

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ABSTRACT

Nanoparticles (NP) are solid colloidal particles ranging in size from 1 to 1000 nm that are utilized as drug delivery agents. The use of NPs to deliver drugs to the brain across the blood-brain barrier (BBB) may provide a significant advantage to current strategies. The primary advantage of NP carrier technology is that NPs mask the blood-brain barrier limiting characteristics of the therapeutic drug molecule. Furthermore, this system may slow drug release in the brain, decreasing peripheral toxicity. This review evaluates previous strategies of brain drug delivery, discusses NP transport across the BBB, and describes primary methods of NP preparation and characterization. Further, influencing manufacturing factors (type of polymers and surfactants, NP size, and the drug molecule) are detailed in relation to movement of the drug delivery agent across the BBB. Currently, reports evaluating NPs for brain delivery have studied anesthetic and chemotherapeutic agents. These studies are reviewed for efficacy and mechanisms of transport. Physiological factors such as phagocytic activity of the reticuloendothelial system and protein opsonization may limit the amount of brain delivered drug and methods to avoid these issues are also discussed. NP technology appears to have significant promise in delivering therapeutic molecules across the BBB.

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INTRODUCTION

Nanoparticles (NPs) are solid colloidal particles, ranging in size from 1 to 1000 nm, consisting of various macromolecules in which therapeutic drugs can be adsorbed, entrapped, or covalently attached. One utility of NPs is to serve as novel drug delivery carriers to tissues throughout the body. This is accomplished by masking the membrane barrier, limiting characteristics of the therapeutic drug molecule, as well as retaining drug stability, with that of the properties of the colloidal drug carrier. This disguising of the drug may allow access across the previously impermeable membrane. Once the NP reaches the desired tissue, release of the drug may occur by desorption, diffusion through the NP matrix or polymer wall or NP erosion, or some combination of any or all mechanisms. Currently, NPs are gaining interest as therapeutic drug carriers across the blood-brain barrier (BBB).

For drugs to be successfully delivered to their target, many factors need to be considered during development of the NP-containing drug. In the case of nanoparticles, not only should these factors be considered for the drug to be released at its target but also with the NP carrier in which the drug is contained through circulation. Table 1 illustrates the requirements for polymeric-based NPs in delivering drug to the central nervous system (CNS). This paper will highlight many of the advantages and limitations found in CNS NP technology.

The BBB represents one of the strictest barriers of in-vivo therapeutic drug delivery. The barrier is defined by restricted exchange of hydrophilic compounds, small proteins, and charged molecules between the plasma and the CNS. The primary mechanism of regulation centers on the anatomical basis of the BBB. The BBB is comprised of a contiguous layer of endothelial cells connected by tight junctions that circumferentially surround the entire cell margin at the brain capillaries. Similar, but not identical, junctions exist at the choroid plexus epithelium and arachnoid membrane. These tight endothelium junctions (*zonulae occludens*) can be ~ 100 times tighter than junctions of other capillary endothelium (1). Thus, the barrier has many of the same properties of a continuous cell membrane, allowing lipid-soluble molecules transport across the membrane whereas hydrophilic solutes demonstrate minimal permeation (2).

While the characteristics of the BBB provide a formidable obstacle for drug therapy in the CNS, they are not insurmountable. Attempts to overcome the barrier in vivo have focused on altering barrier integrity or characteristics, or changing the characteristics of the drug. Figure 1 illustrates strategies utilized to increase CNS drug delivery. Tight junctions at the BBB have been opened by artificially created osmotic pressure and the administration of bradykinin analogs such as RMP-7. Junctional opening of the BBB enables paracellular CNS drug delivery across the barrier. Specifically, Rapoport *et al.* (3) demonstrated that with intracarotid administration of hypertonic arabinose solutions a transient (hours) modification in BBB permeability allowed ≥ 20-fold increase in brain concentrations of hydrophilic compounds. Sanovich *et al.* (4) and others demonstrated increased permeability of the BBB to lanthanum by the administration of the bradykinin analog RMP-7. However, opening the barrier by either mechanism allows CNS entry of toxins and unwanted molecules, potentially resulting in significant damage (5).

Table 1

Ideal Properties of Polymeric-Based NPs for Drug Delivery Across the BBB

Ideal properties of BBB delivery polymeric-based carriers
• Natural or synthetic polymer
• Inexpensive
• Nontoxic
• Biodegradable/biocompatible
• Nonthrombogenic
• Nonimmunogenic
Ideal properties of polymeric-based NPs
• Particle diameter < 100 nm
• Stable in blood (i.e., no opsonization by proteins)
• BBB-targeted (i.e., use of cell surface ligands, receptor-mediated endocytocis)
• No activation of neutrophils
• No platelet aggregation
• Avoidance of the reticuloendothelial system
• Noninflammatory
• Prolonged circulation time
• Scalable and cost-effective with regard to manufacturing process
• Amenable to small molecules, peptides, proteins, or nucleic acids

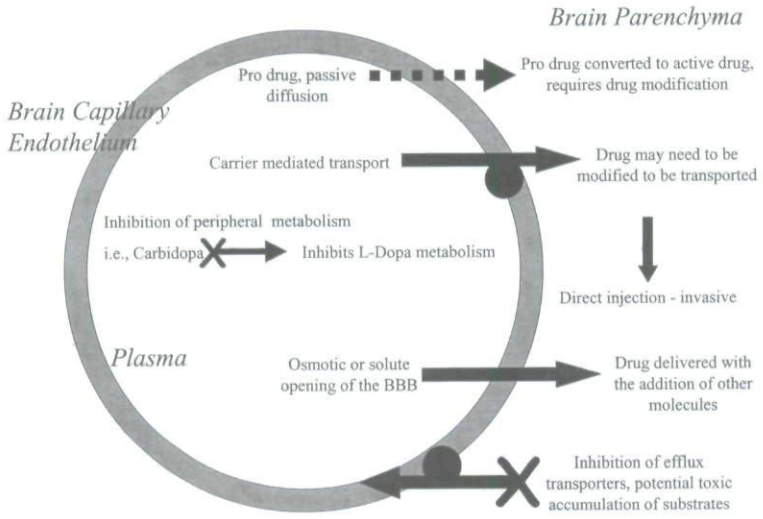


Figure 1. Strategies to increase CNS drug delivery.

Since there are risks associated with changing the permeability of the BBB, attempts have been made to modify drugs to more readily cross the barrier. Pro-drugs are an excellent example of such drug manipulation. With this method, the original compound is manipulated to make it more lipid-soluble, providing greater CNS penetration. While pro-drugs work well, not all compounds (i.e., neurotrophic factors such as glial-derived neurotrophic factor, brain-derived neurotrophic factor, or nerve growth factor) may be manipulated in this way and still maintain therapeutic efficacy. Furthermore, increased lipid solubility may significantly alter pharmacokinetic parameters such that clearance and half-life become undesirable, as is the case with chlorambucil derivatives (6). NPs may be superior to both of these techniques, since no manipulation of the barrier or the drug is necessary.

Another alternative for brain drug delivery is utilization of the native carriers expressed at the BBB. Carriers, also known as transporters, deliver essential hydrophilic and large compounds across the barrier such as choline, purines, amino acids, and lipoproteins. While physiologically expressed to perform transport of needed nutrients, they may be used to deliver drugs to the CNS as well. For example, the chemotherapeutic agent D, L-NAM (for brain tumors) is transported across the BBB by the large neutral amino acid carrier (7). Other drugs also cross the BBB by this carrier, such as baclofen, melphan, sulfoximine, azaserine, and alpha-methyl DOPA (8). While carriers are an attractive means of CNS delivery, the drugs must have carrier-mediated specificity, thus limiting their molecular characteristics. NPs may cross the BBB by passive diffusion or receptor-mediated endocytosis, carrying the drug across the BBB, without requiring drug molecular specificity. However, if high-affinity ligands for these transporters are placed on the surface of the NP, it may be possible to use NPs as a vector for brain or other site-directed delivery.

While these transporters function in the direction of influx from blood to brain, efflux transporters are also present. These efflux transporters (P-glycoprotein, multi-drug resistance protein, and others) are likely located at the BBB for detoxification and/or prevention of nonessential compounds from entering the brain. While the natural effect is beneficial, it is yet another obstacle in delivering drugs to the CNS as many agents that readily cross the BBB are substrates for efflux transporters. CNS disposition of a drug and its metabolites frequently are determined by P-glycoprotein and, furthermore, P-glycoprotein may function as a defense mechanism determining bioavailability and CNS drug concentrations (9). Therefore, while nonselective inhibition of efflux transporters may lead to a therapeutic benefit, there is risk of CNS or peripheral toxicity.

Direct injection into the brain is another approach to circumvent the BBB. This has been accomplished using different techniques. Some examples of direct CNS drug delivery include

delivering nerve growth factor to an Alzheimer's patient by intracerebroventricular infusion into cerebrospinal fluid (10), direct implantation into brain parenchyma of a polymer matrix containing nerve growth factor (11), and transplantation of encapsulated cells which secrete nerve growth factor into forebrain neurons (12). While these techniques can be successful to achieve certain therapeutic goals, disadvantages exist for direct injection for this method. The primary disadvantage is the requirement of extremely invasive neurosurgery. This limits the potential to treat only gravely ill patients and then only if the affected area is accessible. Furthermore, diffusion of the drug from the injection site may limit therapy.

While direct drug injection into brain may be a somewhat viable method of circumventing the BBB (considering the limitations described above), there are other strategies as well. For example, intracarotid infusion delivers the drug directly to the BBB, increasing the concentration gradient and resulting in increased brain concentrations. However, intracarotid infusions pose major risk of injury and, due to drug streaming, variable brain concentrations are seen (13).

The use of NPs as colloidal drug carriers may have a decided advantage over the previously mentioned approaches to circumvent the BBB. In this review, we will discuss NPs in relation to delivery across the BBB, the primary methods of preparation, NP characterization, current research published on NP brain delivery, and the reticuloendothelial (RES) system as an obstacle to CNS delivery.

MANUFACTURING METHODS

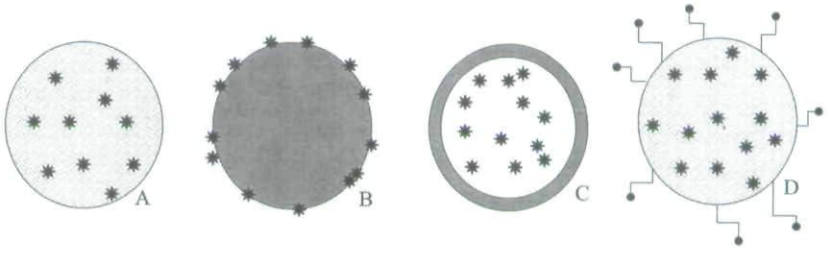
NPs vary in types of polymers, stabilizers and surfactants used in their manufacturing process. Each excipient added may have a significant affect on brain drug uptake, drug distribution throughout the body, and persistence of the drug in the plasma. When manufacturing NPs as drug carriers, in-vivo and in-vitro testing should consider the factors listed in Table 1. Primary manufacturing methods include (14):

1. emulsion polymerization
2. interfacial polymerization
3. desolvation evaporation and
4. solvent deposition.

Various NP structures may result secondary to each manufacturing method. Furthermore, drug loading can be accomplished by absorption, adsorption, and encapsulation (Fig. 2).

Emulsion Polymerization

Emulsion polymerization, which characterizes both radical and anionic polymerization, is one of the most frequently used techniques for production



A. Solid colloidal NP with homogenous dispersion of drug
B. Solid NP with drug associated on exterior
C.Nanocapsule with drug entrapped
D. Solid colloidal NP with homogenous dispersion of drug and cell surface ligand

Polymeric matrix
Drug
Cell surface ligand
Solid NP

Figure 2. Various types of drug-loaded nanoparticles for CNS delivery.

of NPs. The process consists of building a chain of polymers, which acts as the drug carrier, from single monomer units of a given compound. Polymerization occurs spontaneously at room temperature after initiation by either free radical or ion formation. Triggers for polymer growth include high-energy radiation, UV light, or hydroxyl ions. Once polymerization is complete, the solution is filtered and neutralized to remove any residual monomers. The polymers form micelles and droplets (NPs), consisting of approximately 100 to 10^7 polymer molecules. The mass of polymers inherent in this type of NP formulation provides the available space that acts as a carrier for adsorption or absorption of the drug (Fig. 2A and B) (15).

Emulsion polymerization has numerous advantages in NP formulation. It is rapid compared with other methods, stabilizers and surfactants are generally not needed, and it is easily scaled up for large manufacturing requirements (16).

Emulsion polymerization can also be accomplished in an organic phase rather than an aqueous phase. This process has been adapted for use with polyakyl-cyanoacrylate NPs.

The primary disadvantages of emulsion polymerization is its requirement for free radicals, radiation or UV light to trigger polymerization. The necessity of these triggers precludes the incorporation of peptides and proteins during polymerization (Table 1). Hence, in order maintain the stability of incorporated proteins or peptides the NPs must be purified via dialysis and centrifugation to remove residual monomers. Purification requirements limit the ability of this process to be scaled up for large manufacturing. Furthermore, emulsion polymerization also has the disadvantage that it requires large amounts of organic solvents and thus has the potential for toxicity (17).

Interfacial Polymerization

Interfacial polymerization is similar to emulsion polymerization in that monomers are used to create polymers. However, the mechanism is different. Interfacial polymerization occurs when an aqueous and organic phase are brought together by homogenization, emulsification, or micro-fluidization under high-torque mechanical stirring. This precludes the inclusion of peptides/proteins at this step secondary to mechanical shearing. For example, the creation of polyalkylcyanoacrylate nanocapsules (Fig. 2C) was completed when the monomer was dissolved in oil and slowly added through a small tube to an aqueous phase with constant stirring. The monomer then spontaneously forms 200–300 nm capsules by anionic polymerization. Drug incorporation was accomplished by adding the drug with the monomer in the organic phase. This encouraged the drug to be enveloped in the matrix of the NP (18).

A subset of interfacial polymerization is the process of adding a solvent mixture of benzyl benzoate, acetone, and phospholipids to the organic phase containing the drug and monomer. It has been suggested that this process encourages the formation of the nanocapsule shell between the aqueous phase and the benzyl benzoate drops in the organic phase (19). One advantage of interfacial polymerization may be the encapsulation of the drug. Once the drug is encapsulated, it is protected until it reaches the target tissue and degradation occurs. In the case of CNS delivery, it is desirable to protect or disguise the drug until it is past the barrier and can be released into the brain.

Denaturation and Desolvation

Macromolecules such as albumin and gelatin can also be used in the production of NPs. Using such macromolecules capitalizes on the natural affinity between the macromolecule and drug. Two primary processes—oil denaturation and desolvation—are used to process macromolecules as NPs. Oil emulsion denaturation is the process by which large macromolecules are trapped in an organic phase by homogenization. Once trapped, the macromolecule is slowly introduced to an aqueous phase undergoing constant stirring. The particles formed by the introduction of the two immiscible phases are then hardened by crosslinking with an aldehyde (20) or by heat denaturation (21). Unlike the above preparation methods, the quantity of the macromolecules, temperature, and emulsification time have little effect on the resultant particle size. The greatest effect seen on this property is the type of oil used (15).

Macromolecules may also form NPs by "desolvation." Desolvation occurs when the macromolecule is dissolved in a solvent where macromolecules reside in a swollen, coiled conformation. The swollen macromolecule is then induced to coil tightly by changing the environment pH, charge, or the use of a desolvating agent such as ethanol. The macromolecule may then be fixed and hardened by

crosslinking to an aldehyde. Drugs bound to the protein or macromolecule, prior to the crosslinking step, become entrapped in the newly-formed particle. The major drawback of this method is that the quantities of NPs and the drug absorbed are very low compared with other methods (22).

Solid lipid NPs are created by high-pressure homogenization. Solid lipid NPs share the same benefits of fat emulsions and liposomes while avoiding some of the respective drawbacks. Solid lipid NPs may be sterilized and autoclaved similar to fat emulsions (23) and possess a solid matrix that provides a controlled release, avoiding the burst release seen with fat emulsions (24).

Nanoparticle Drug Release

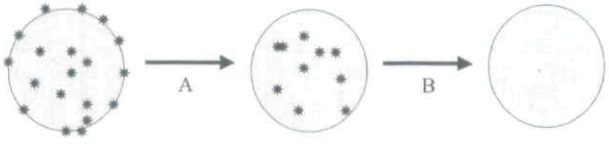
Figure 3 illustrates the pharmacokinetic analysis of the release of doxorubicin from NPs, which was characterized by Gupta *et al.* (25) as a bi-exponential equation:

$$C_t = Ae^{-\alpha t} + Be^{-\beta t}$$

where C is the concentration of drug remaining in the NP at a given time; A and B are system-characteristic constants, intercepts of release; α is the initial rate constant; and β is the secondary rate constant.

This model suggests there is an initial rapid removal of the drug from the NP, possibly related to early loss of drug loosely associated on the surface of the NP (Fig. 4). Once the rapid component of release is complete, there is a slower, much more controlled release of the drug owing to either NP degradation or diffusion of drug through the NP matrix or shell. This latter release has been characterized by both zero-order and first-order kinetics (25). Zero-order kinetics tend to occur with biodegradable NPs, whereas first-order release occurs with nonbiodegradable NPs. Regardless of the kinetics of drug release, it appears release is dependent on NP degradation or erosion of nanocapsule shell (15).

Drug release is also dependent on the structure of the NP. Drugs may be incorporated in a solid lipid NP, encapsulated in an NP shell, adsorbed onto the surface, or crosslinked to the NP. These factors, plus the type and length of the polymer, can have significant effects on the release of the drug (15).



A.Initial rapid desorbtion of drug from surface of nanoparticle known as burst effect. May provide initial dose of drug.

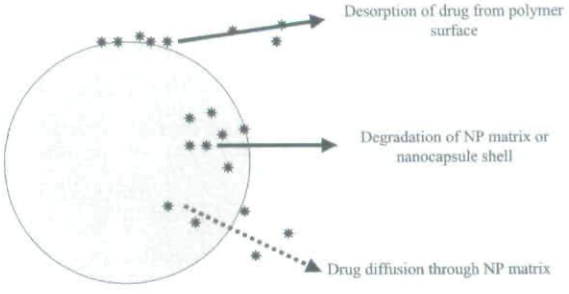
B.Slow controlled release of drug. Characterized by both first- and zero-order equations. Release is dependent on NP degradation or erosion of nanocapsule shell.

Figure 3. Pharmacokinetic analysis of drug release from NP.

NANOPARTICLE CHARACTERIZATION

The primary characterization of NPs is the size of the newly formed particle. Several physiochemical factors may influence the size of NPs. Factors such as pH of the solution used during polymerization, amount of initiation triggers (heat, radiation etc.), and concentration of monomer units may affect NP size in the 100–200 nm range (26,27). However, the density, molecular weight, hydrophobicity, surface charge, and surface morphology may also be helpful in predicting drug delivery to specific targets. Kreuter (15) reviewed the primary methods of determining the physical and chemical properties of NPs.

Sizing is one of the most important steps in the characterization of the prepared NP. Photon correlation spectroscopy using light scattering is one of the most common methods of sizing. Photon correlation spectroscopy relies on Brownian motion, which predicts that smaller particles have increased motion in solution. By illuminating the particles with a laser beam and analyzing the time dependency of the light changes, the NP can be accurately



Mechanisms of NP drug release may be associated with one or a combination of any or all proposed mechanisms.

Figure 4. Mechanisms of drug release from NPs.

sized. Characterizations of molecular weight, density, and crystallinity can be accomplished by gel chromatography, helium compression pycnometry, and x-ray diffraction, respectively (15).

MECHANISMS OF NANOPARTICLE TRANSPORT ACROSS THE BBB

Transport of NPs across the BBB has been characterized similarly to many of the known transport mechanisms described for other drugs (Fig. 5). Passive diffusion at the BBB occurs when a drug dissolves in the lipid membrane of cerebrovascular endothelial cells and is then released into the brain. Passive diffusion, which depends on lipophilicity of the drug, charge, concentration gradient, molecular weight, and degree of protein binding, is characterized by Fick's law of diffusion:

$$-\mathrm{d}C/\mathrm{d}t = k(C_1 - C_2)$$

Transport of drugs across the BBB is dependent on carrier proteins at the capillary endothelial cells. Carrier-mediated transport at the BBB can occur as facilitated transport along the concentration gradient, active transport (against the concentration gradient), and endocytosis. Carrier-mediated transport is analyzed with Michaelis–Menten saturation kinetics, where

$$\text{Rate} = \frac{V_{\max} * \mathrm{C}}{(K_{\mathrm{m}} + \mathrm{C})} + kd$$

The transport of NPs across the BBB has been hypothesized to occur by receptor-mediated endocytosis and/or passive diffusion.

Passive Diffusion

The effect of lipid coating polysaccharide NPs and its effect on transport across an in-vitro BBB (bovine brain capillary endothelial cells) was evaluated by Fenart *et al.* (28). These authors compared uptake of polysaccharide NPs, crosslinked with phosphate (anionic) and quaternary ammonium (cationic) ligands, with and without a surrounding lipid bilayer. They demonstrated that when a lipid bilayer containing dipalmitoyl phosphatidyl choline and cholesterol coating is applied to the charged NPs, a 3–4-fold increase in brain uptake was observed. Furthermore, they showed the NP remained intact as it crossed the BBB and transport was not due to altered BBB integrity. They also demonstrated that albumin, a large protein normally precluded from brain distribution, had a 27-fold increase in uptake when coated with the same lipid bilayer. However, in the presence of erythrocytes, a significant decrease in transport was seen, possibly due to an NP–erythrocyte interaction.

The use of NPs for drugs that demonstrate permeation of the BBB by passive diffusion in the free state may improve the drug's brain distribution profile. This was most notably demonstrated with amitriptyline by Shroeder *et al.* (29). After

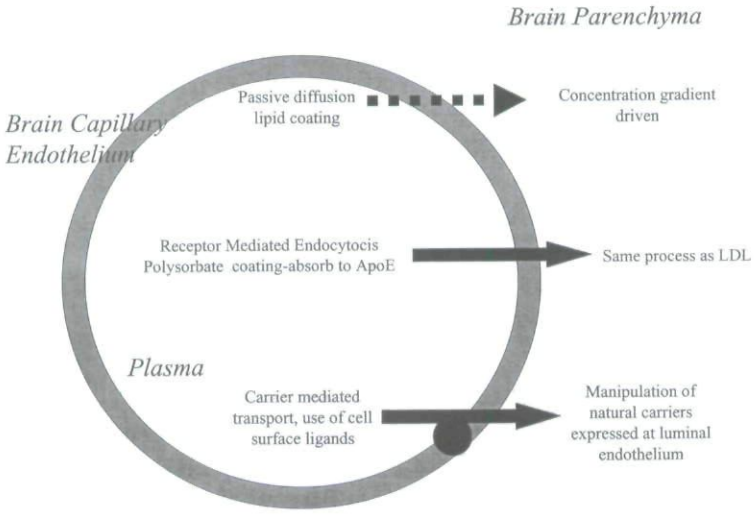


Figure 5. Mechanisms of nanoparticle CNS entry.

amitriptyline was adsorbed onto polybutylcyanoacrylate NPs, using the stabilizer dextran-70,000 and polysorbate-80 as a surfactant, a 10-fold increase in brain concentrations was found. The authors hypothesized the increase was secondary to an enhancement of the plasma concentration, resulting in a larger gradient at the BBB and thus greater concentrations of the drug entering the brain by passive diffusion. Furthermore, NP degradation products may act as adsorption enhancers (30), leading to increased passive diffusion.

Receptor-Mediated Endocytosis

The transport mechanism of labeled polybutylcyanoacrylate NPs coated with polysorbate-80 across the BBB has been suggested to be cellular endothelial endocytosis. NPs were administered intra-arterially and localized by transmission electron microscopy and fluorescence microscopy. When the NPs were not coated with surfactants, the particles remained in the blood vessels (31). Borchardt *et al.* (32) confirmed this finding using [^{14}C]–labeled NPs in brain microvascular endothelial cells. Furthermore, uptake of NPs coated with polysorbate-80 was inhibited by the phagocytic inhibitor cytochalasin B (33).

It has been suggested that apo-E adsorbs onto NPs coated with polysorbate-20, 40, 60, or 80. A logical conclusion is that polysorbate-coated NPs be subject to the same endocytotic process low-density lipoproteins undergo at the BBB (34).

Multiple mechanisms for transport of NPs have been described. Whereas, initially, these mechanisms may seem contradictory, many factors may influence the BBB transport mechanism, including the type of polymers, size of the NP, types of surfactants, and the drug molecule itself. Further studies should evaluate the mechanisms responsible for NP BBB transport, considering each of these factors.

NANOPARTICLES LOADED WITH ANESTHETICS

Earlier studies in the use of NPs to improve brain drug distribution involved anesthetic agents such as dalagrin, kytorphin, and the neuromuscular blocking agent tubocurarine (Table 2). These anesthetics were chosen because they exhibit therapeutic effects when given directly in the brain, but with peripheral administration no anesthesia was seen. This suggests these anesthetics do not cross the BBB appreciably from the plasma.

Polybutylcyanoacrylate NPs coated with polysorbate-80 have been shown to deliver the polypeptide dalagrin across the BBB after intravenous injection. Significant anesthesia was noted when dalagrin is injected directly into the brain, whereas with peripheral administration no anesthesia was observed. This suggests dalagrin does not cross the BBB. When dalagrin was adsorbed onto the polybutylcyanoacrylate NPs coated with polysorbate-80 as a surfactant, significant and prolonged analgesia took place following intravenous administration. After anesthesia was induced, the central acting opiate antagonist naloxone was administered peripherally, causing a blockade of the analgesia effects. This suggests the dalagrin-induced anesthesia is mediated by central mechanisms. Furthermore, when dalagrin is adsorbed onto polybutylcyanoacrylate without the surfactant coating, no analgesic effect was seen (30, 35). Both studies indicate that polybutylcyanoacrylate NPs with the surfactant can aid in the delivery of drugs across the BBB.

In addition to dalagrin, studies have been conducted to evaluate the analgesic effects of kyotorphin-loaded NPs (35). It produced central analgesic effects, but only when the particle was stabilized by dextran 70 kDa.

Anesthetics previously demonstrated to be excluded from brain were shown to cross the BBB by use of nanoparticles (36). These studies assessed adsorbed tubocurarine (a quaternary ammonium compound that has minimal BBB permeation) onto polybutylcyanoacrylate particles coated with polysorbate-80 and found this complex to be transported at BBB. Tubocurarine, when given intravenously, is a myoparalytic found in minimal concentrations in the cerebrospinal fluid. It does not affect spontaneous and evoked bioelectric activity of the brain when given intravenously. However, when combined with NPs as described above and administered peripherally, seizure electroencephalograph patterns were noted in the animals. This study demonstrates the potential of specific NPs to carry charged cations across the BBB (36).

NPs have been demonstrated to transport charged anesthetic agents across the BBB. Furthermore, these agents produce similar therapeutic effects when given intravenously as the effects originally observed when administered directly into

Table 2

Examples of NPs Used for Delivery of Drugs Across the BBB

Drug Tested (Ref No.)	NP type	Polymer Used/Stabilizer	Surfactant Type	NP Size (nm)	Results
Camphotericin (39)	Solid lipid NP	Soybean oil	Poloxamer 188	196.8	Increased brain AUC–10.4 fold
Albumin (28)	Polysaccharide core	Maltodextrin	Lipid coating—dipalmitoyl phosphatidylcholine	60	27-fold increase in transport across in-vitro BBB model
Dalagrin (35)	Solid NP	Polybutylcyanoacrylate	Polysorbate-80	260	Analgesia study, increased latency by 50%
Valproic acid (46)	Solid NP	Butylcyanoacrylate/dextran 70 kDa, polysorbate-85	Polysorbate-80	Not evaluated	No increase in brain concentrations
Dalagrin, Kytorphin (29)	Solid NP	Polybutylcyanoacrylate/ dextran 70 kDa, polysorbate-85	Polysorbate-80	Dextran: 288 Poly: 80–195	Analgesia study: increased latency by ~50%
Amitriptyline (29)	Solid NP	Polybutylcyanoacrylate/dextran 70 kDa, polysorbate-85	No coating	Dextran: 288 Poly: 80–195	Increased brain AUC > 50%
Doxorubicin (47)	Solid lipid NP	Stearic acid	Epikuron 200	90	Levels ~ 1/4 of plasma after 4 hr vs. zero in brain without NP carrier
Dalagrin (31)	Solid NP	Polybutylcyanoacrylate/ dextran 70 kDa	Polysorbate-80	230	Analgesia study: increased analgesia effect by ~50%
Tubocurarine (36)	Solid NP	Butylcyanoacrylate/dextran 70 kDa	Polysorbate-80	230	Epileptiform spikes on EEG
Doxorubicin (37)	Solid NP	Butylcyanoacrylate/dextran 70 kDa	Polysorbate-80	270	~ 6 mg/g (brain) at 2–5 hr, vs. zero without carrier
Radiolabeled NPs (32)	Solid NP	Poly-methylmethylacrylate	Poloxamer 338 Polaxamine 908 Polaxamer 188 Polaxamer 407 Polysorbate-80	Not evaluated	No uptake increase (BMEC) No increase ~10% increase ~17.5% increase ~15.1% increase

the brain. The choice to use anesthetic agents to assess NP transport is simplistic. Transport is determined by induction of anesthesia. However, one must also consider whether the proposed anesthetic effect is from therapeutic efficacy, simply that of toxicity, or a mixture of both. All of the above studies are short-term terminal studies and one must be cautious in extrapolating application to acute usage in humans.

NANOPARTICLES LOADED WITH CHEMOTHERAPEUTICS

Tumors within the brain have provided unique therapeutic challenges. Many of the chemotherapeutic drugs are polar molecules and do not readily penetrate the BBB. This is further complicated by the need to maximize time and exposure concentration of the chemotherapeutic agent to the cancer cells. However, when these two factors are maximized to provide therapeutic efficacy, plasma concentrations are high, resulting in significant systemic toxicity. NPs as chemotherapeutic carriers have been studied as a solution to these issues (Table 2).

Doxorubicin, an anthracycline antibiotic, is a chemotherapeutic agent that intercalates into DNA, resulting in an inhibition of DNA synthesis. Doxorubicin is a polar molecule that does not normally cross the BBB. When doxorubicin was given intravenously adsorbed on polybutylcyanoacrylate NPs with polysorbate-80 as a surfactant, CNS doxorubicin concentrations were therapeutic at ~6 μg/g in the brain (37). Furthermore, the NP containing doxorubicin administered intravenously to rats with intracranially transplanted glioblastomas led to a cure in ~40% of these rats. In contrast, of the rats that received free doxorubicin, only one survived in seven control groups (34,38).

When the lipophilic anticancer drug camphotericin was adsorbed on solid lipid NPs, in-vitro drug release of up to 1 week in mice. The area under the curve and the mean residence time of camphotericin-solid lipid NPs were higher than control, most notably in brain, heart, and reticuloendothelial cell containing organs. Also, adsorbing of camphotericin on solid lipid NPs resulted in protection of the more effective lactone form of the drug from hydrolysis to the carboxalate form. Solid lipid NPs may be a promising sustained-release and drug-targeting system for lipophilic CNS antitumor drugs (39).

The delivery of antitumor drugs by NPs is a promising alternative to surgery and direct injection of drugs in the CNS. One significant benefit of tumor therapy with NPs as a drug carrier is the prolongation of mean residence time in the body. Whereas this benefit may increase the exposure of the tumor to the chemotherapeutic agent, it also prolongs exposure of the remainder of the body to the drug, potentially increasing toxicity.

RETICULOENDOTHELIAL SYSTEM: AN OBSTACLE FOR CNS DRUG TARGETING

Initially, targeting NPs to the brain proved unsuccessful when given intravenously. Failure of NPs to reach the CNS in appreciable quantity was due to NP uptake by the RES, also known as the mononuclear phagocytic system. The RES is a collective group of mononuclear cells originating from bone marrow that have phagocytic responsibility in removing small foreign particles from the vascular space. While the cells are found throughout the body, a high number of cells are localized in the liver (Kupffer cells), spleen, and bone marrow. The RES significantly removes a large portion (up to 80–85%) of NPs from the vascular space, limiting the exposure of NPs at the cerebrovasculature and resulting in decreased drug concentration in the brain (40).

Strategies to overcome RES uptake include external guidance of magnetically responsive NPs and NP coating with antibodies or hydrophilic surfactants. Magnetic guidance consists of manufacturing NPs containing magnetite (Fe_3O_4) and using an external magnet. For example, doxorubicin was incorporated into these 'magnetic' NPs (41). When a magnet (3000 gauss) was placed near the rat-tail, a 24-fold increase in the area under the curve was observed in comparison to free drug. This has been repeated in NP targeting to the brain. Pulfer and Gallo (42) used similar magnetic NPs without drugs in an attempt to deliver drugs to the brain. Rats were given intracarotid injections of the NPs and a magnetic field applied to the brain area and compared with control. After sacrificing the animals at 30 min and 6 hr, brain magnetite concentrations were determined by atomic absorption spectroscopy. Magnetic fields resulted in increased brain concentrations and decreased nontarget tissue

concentrations. While efficacy of drug delivery has been shown by this novel technique, it may be impractical for use in human subjects. Issues related to this technique for human use include duration of magnetic force necessary to exert the effect, chronic force effects, chronic toxicity of this NP and its metabolites (notably magnetite), as well as compliance by sick patients.

Another solution to the problem of rapid uptake of NPs by the RES is coating with surfactants. Primary surfactants used include polaxamine 908 and polysorbate-80. Reducing the uptake of NPs by the liver and other organs of the RES will result in increased residence time of the drug in circulation and increased uptake in non-RES organs. Polaxamine 908 used as a surfactant on hydrophobic NPs was shown to reduce RES uptake in the liver when compared with uncoated drug (72% vs. 19%). There was also a significant reduction of NP uptake by the spleen, lungs, and bone marrow (14).

Polysorbate-80 has been shown to be effective in minimizing uptake by organs that localize RES (43). When NPs containing bound doxorubicin were administered intravenously, with and without surfactant, significant differences were found. The plasma half-life of doxorubicin increased approximately fourfold compared with free drug administration. Furthermore, it was noted that greater concentrations of the drug were seen in the RES organs using uncoated and coated NPs when compared with free drug. Coating NPs with surfactants significantly reduced the quantity of drug in these organs (37). Altering the surface characteristics of NPs with surfactant increases the ability of NPs to reach the BBB, without increased uptake by the RES.

A concern regarding polysorbate-80 coated polybutylcyanoacrylate NPs is toxicity. There is conflicting evidence of lack of toxicity and toxic effects occurring when this surfactant and polymer is used. Olivier *et al.* (44) hypothesized that dalagrin adsorbed onto NPs of polybutylcyanoacrylate coated with polysorbate-80 and administered at 166 mg/kg resulted in death in 3 of 10 subjects. The surviving mice had significantly decreased activity, after a short burst of hyperactivity and obvious discomfort. The authors suggested this toxicity is mediated by rapid esterase biodegradation of the polybutylcyanoacrylate polymer to toxic compounds. Furthermore, tests in an in-vitro model of the BBB showed increased permeability to sucrose, a vascular integrity marker, suggesting a compromise in the barrier by polybutylcyanoacrylate-coated NPs. Kreuter refutes the toxicity suggested by the Olivier study. It was argued that there was no CNS toxicity since the normal response to dalagrin, an opioid, is a short hyperactive burst of activity followed by decreased activity. In addition, the BBB opening was seen in vitro and not in vivo, and multiple other authors have not observed in-vitro BBB opening (35).

In summary, phagocytic activity of the RES presents a major obstacle in delivering NPs to the brain. Some investigators circumvented this problem by manipulating NP content (magnetite) or adding surfactants (poloxamine and polysorbate-80). Although all studies have shown an increase in brain drug delivery, there may be a concern regarding the toxicity of these novel preparations. Further studies should explore the mechanisms of toxicity, LD_{50}s, and practicality of use in human subjects.

CURRENT CLINICAL USE OF NANOPARTICLES

Currently, the only drug marketed using polymeric NPs is the diagnostic agent Abdoscan® by Nycomed. Abdoscan is a colloidal NP containing crystalline superparamagnetic iron oxide particles stabilized with low molecular weight dextran. The primary use of this novel NP is for diagnostic imaging of primarily spleen and liver tumors. Abdoscan takes advantage of the NP phagocytosis process that occurs in RES organs. Phagocytic uptake of colloidal NPs results in increased magnetic resonance imaging in the organ. Since tumor cells are not capable of phagocytosis, there is no enhanced imaging in the tumor and a sharp contrast is produced between healthy and tumor tissue. However, at this time there are no marketed products using polymeric NPs for drug delivery across the BBB (45).

CONCLUSIONS

After 30 years of research on polymeric NPs, this delivery system practically does not exist clinically, yet NPs appear to have significant potential in delivering drugs to the brain. It has been demonstrated that NPs can cross the barrier intact by passive diffusion and receptor-mediated endocytosis. Further, site-directed brain delivery of NPs may be

possible by the use of high-affinity NP surface ligands to native BBB transporters. Once the BBB-transporter-targeted NP is in brain, a slow controlled release of the drug occurs, targeting CNS tissue and avoiding other organs, which should reduce peripheral or systemic toxicities.

Currently, areas of research have focused on anesthetic and chemotherapeutic agents. The use of anesthetics is a good choice for basic research determination of NP crossing the BBB. However, there is no clinical need for novel anesthetic agent brain delivery. The study of NP-loaded chemotherapeutic agents target the therapeutic problem of cancer in the CNS. The majority of chemotherapeutic agents do not cross the BBB, and ones that do are removed by the efflux protein, p-glycoprotein. NPs appear to increase the brain area under the curve of both doxorubicin and camphotericin. Further studies should consider the evaluation of other CNS diseases, which are limited in therapeutic treatments by the BBB.

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