



DRUG DELIVERY TO BRAIN AND BONE MARROW: A REVIEW

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ABSTRACT

The available figures for central nervous system (CNS) pathology has demonstrated that approximately 1.5 billion people undergoing from disorders of CNS. The most distressing fact about delivery of drugs to the CNS is the presence of blood brain barrier (BBB) that have a tendency to impair the drug distribution and it denotes the major impediment for the development of CNS drugs. Despite great strides in the basic science of brain physiology and disease in the past decade, delivery issues have received minimal attention. Current estimates are that 98% of all small molecule drugs minimally cross the BBB, and miniscule amounts of large molecule drugs cross the BBB, except leakage in areas of BBB dysfunction. The bone marrow is a prime haematopoietic organ. Bone marrow targeted drug delivery systems appear to offer a promising strategy for advancing diagnostic, protective and/or therapeutic medicine for the hematopoietic system. Therapeutic approaches might include gene therapy, selective delivery and local release of antimicrobials, as well as agents that could induce self-renewal, proliferation, and maturation of stem and progenitor cells. The key to such approaches, however, may reside in the distinct physiological function of the major vascular constituents of the bone marrow, the so called sinuses. In light of all the above mentioned possibilities this review has been designed to understand the various measures of drug delivery across BBB and bone marrow.

KEYWORDS: Blood brain barrier; Bone marrow; Liposomes; Nanoparticles.

1. INTRODUCTION

The delivery of drugs to CNS is a challenge in the treatment of neurological disorders. Drugs may be administered directly into the CNS or administered systematically for targeted action in the CNS. BBB which limits the access of drugs to the brain substance, is the major challenge to CNS drug delivery. Various strategies that have been used for manipulating the BBB for drug delivery to the brain include osmotic and chemical opening of the BBB and also the use of transport/carrier systems. Other strategies for drug delivery to the brain involve bypassing the BBB. Various pharmacological agents have been used to penetrate the BBB and direct invasive methods can introduce therapeutic agents into the brain substance. It is important to consider the net delivery of the agent to the CNS and the ability of the agent to access the relevant target site within the CNS.^[1] It has been estimated that more than 98% of CNS active drugs of synthetic origin are unable to cross the BBB sufficiently to achieve desired therapeutic drug concentration. Also, majority of the small molecule drugs and almost all of the large molecule drugs, including biotechnology based products are unable to cross blood brain barrier.^[2] The ongoing research in the intranasal route of administration in

recent years has shown potential for delivery of drugs to brain. The nose-to-brain drug delivery of drugs is advantageous as it requires low dose of drug and also avoids first pass effect. Also it is fast in action and suitable for the drugs that degrade in gastrointestinal tract (GIT). Nose-to-brain delivery also avoids BBB which is important factor to be considered in formulation of CNS targeting drugs. This route of administration is also non-invasive, painless and proves to be useful in emergency conditions. Also, research related to Neurodegenerative disorders are on progress.^[3] In adult human the place for production of hematopoietic stem cells (HSCs) from which all blood cells are derived is the bone marrow. It serves as the only permanent hematopoietic organ in human.^[4] It lies within the trabecular bone. Bone marrow stroma and trabecula supports and maintains the hematopoietic tissue. Stroma consists of osteocytes, adipocytes, reticular cells, vascular endothelium and extracellular matrix. Extracellular matrix is composed of collagen, proteoglycans, glycosaminoglycans and adhesive proteins. The adult human bone marrow normally produces 2.5 billion red blood cells (RBCs), 2.5 billion platelets and 1 billion granulocytes per kilogram of body weight per day.^[5] The bone marrow stroma also contains mesenchymal stem cells (MSCs) that are multi-potent

adult stem cells that can differentiate into a variety of cell types like osteoblasts, chondrocytes, myocytes, adipocytes, etc. They can also trans-differentiate into neuronal cells. They support the survival and the proliferation of hematopoietic stem cells (HSCs). Clinically, MSCs may be used to enhance HSCs engraftment after transplantation, to correct inherited disorders of bone and cartilage or as vehicles for gene therapy.^[6] In case of bone marrow, the research in the carrier involved delivery studies has mainly focused on targeting. A few studies have been performed to target drugs to hard tissues. In these studies, Alizarin Red S, tetracycline, calcein and bisphosphonates have been applied for their strong affinities to hydroxyapatite (HA). HA is the major inorganic component of human bone and teeth tissues.^[7] Tetracycline and its analogues were linked and bisphosphonates were conjugated to different macromolecules (protein, PEG1) and low molecular weight compounds to increase their stability, solubility and their targeting properties. Glutamic acid and aspartic acid peptides were reported as bone-targeting moieties to deliver drugs to the bone.^[8]

2. ROLE OF THE BBB IN DRUG DELIVERY TO THE BRAIN

The net uptake of a drug by the brain via the BBB depends on the overall difference between the uptake and efflux processes (Fig. 1) ^[9]. The uptake is controlled by several factors, including the systemic disposition of the drug and the properties of endothelial cells. The systemic disposition is critical because it controls the amount of drug available for crossing the BBB. The main parameters are the area under the blood–time curve and the maximal systemic concentration, which determines the blood–brain concentration gradient. Binding to plasma proteins may sometimes restrict drug uptake by the brain.^[10] Similar properties determine the efflux or secretion of drugs from the brain extracellular fluid to the blood via the BBB, although the absence of protein from the brain ECF means that drugs are not bound to protein. Therefore, the permeability of endothelial cells and their capacity to metabolize drugs actively controls the amount of drug crossing the BBB in both directions. Endothelial cells contain several enzymes implicated in drug oxidation and conjugation and these may interact with drugs.^[11] Permeability is controlled by several properties of the endothelial cells. The paracellular movement of drugs does not occur due to the presence of tight junctions linking the endothelial cells, but small lipophilic drugs (<600 Da) may enter the brain by penetrating the lipid membrane of the endothelial cells. The passive diffusion of a drug depends on its blood/brain concentration gradient and its lipid solubility, but it is inversely related to its degree of ionization and its molecular weight.^[12] Most of these trans-membrane proteins are in the luminal or abluminal membranes of the endothelial cells and control the uptake of numerous drugs. Thus, many amphipathic cationic drugs are carried by at least one ABC protein, the P-glycoprotein at the luminal pole of the BBB.^[13]

Non-diffusible small molecules, such as hydrophilic compounds, peptides and proteins, can cross the BBB via the endothelial cells by carrier-mediated transport or endocytosis. The BBB is highly specialized and transport across it is often asymmetric; it depends on ion channels, specific bidirectional transporters, energy-dependent pumps and a limited amount of receptor-mediated endocytosis.^[14] Receptor-mediated transport systems work via endosomes; substrates are taken up into endosomal sacks by energy-linked systems and then transported from the brain extracellular space to the inside of cells or in the reverse direction. The compounds must use pinocytotic vesicles and adsorption-mediated or receptor-mediated transcytosis.^[15]

2.1. DRUG DELIVERY ACROSS THE BBB

Simple diffusion of a neuro-therapeutic is enhanced by both a high degree of lipophilicity and a molecular weight less than 400–500 Da. Developing in-silico predictors of the chemical characteristics required for diffusion across the BBB has been the main strategy used by pharmaceutical companies to predict the ability of novel compounds to penetrate into the CNS.^[16] These predictors indicate that size and solubility restrictions of diffusion prevent brain uptake of many drugs including large and hydrophilic molecules, proteins and antibodies. Additionally, efflux transporters remove many lipid-soluble, small drugs from the endothelial cell, preventing distribution into the brain.^[17] Strategies have been developed to bypass the BBB via invasive techniques, such as direct injection into the brain, infusion or global disruption of the BBB.^[18] Disruption of the BBB by hypertonic mannitol,^[19] alkylglycerol^[20] or a bradykinin analogue^[21] increases BBB permeability, but may cause permanent brain damage from unintended molecules entering the brain. Obviously, these approaches cannot be used globally in designing novel neuro-therapeutics. The use of less-invasive nanoparticles and liposomes as drug-delivery vehicles to the CNS have also received recent attention and are reviewed elsewhere.^[22] Knowledge on the localization of these transporters in either the luminal or abluminal membrane of the endothelial cell provides opportunities to develop novel drugs that directly target these transport systems. Importantly, however, the efflux transport systems must also be taken into consideration when rationally designing drugs targeting facilitated transporters as substrates of these transporters are often also targets of efflux transport systems.^[23]

2.2. STRATEGIES FOR DELIVERY OF DRUG ACROSS THE BBB

2.2.1. CARRIER-MEDIATED TRANSPORT (CMT)

CMT provides a facilitated mechanism for certain small molecules, nutrients and hormones to passively cross the BBB following a concentration gradient. CMT proteins include the organic anion-transporting polypeptides, the large neutral amino acid, mono carboxylic acid and glucose organic anion and cation transporters. CMT

transporters are substrate-selective and carry their substrates via uni- or bi-directional, mono-, co- or counter-transport. The rate of substrate transport depends on substrate affinity for the transporter, molecular driving force, intra - cellular binding capacity and, in some instances, efflux transport activity as these substrates are often also targets of efflux transport systems.^[24,25] To exploit CMT systems that could facilitate drug delivery into the CNS, researches have been primarily focused on structural similarities between therapeutic drug molecules and endogenous transporter substrates.^[26] Molecular cloning of transporters allows the determination of quantitative structure–activity relationships (QSARs) for individual transport systems. This knowledge, in turn, facilitates the rational design of novel small-molecule therapeutics against a target within the CNS and a specific carrier-mediated transporter. Trojan horse drugs can also be designed to target specific CMT systems.^[27]

2.2.2. LARGE NEUTRAL AMINO ACID TRANSPORTER (LAT1)

The LAT1 is a nutrient transporter that is located in the luminal and abluminal membranes of the brain capillary endothelium.^[28] At the BBB, LAT1 mediates brain uptake of neutral l -amino acids such as phenylalanine, leucine and tyrosine.^[29] Therefore, LAT1 has received attention for delivering drugs into the brain that are either similar in structure to LAT1 substrates or are conjugated to LAT1 substrates. The most successful example of the former approach is l-DOPA (3,4-dihydroxy-l-phenylalanine), an amino acid derivative that is used to increase brain dopamine levels in Parkinson's disease. While dopamine itself does not cross the BBB, LAT1 facilitates l-DOPA transport into the brain, where it is decarboxylated to active dopamine.^[30]

2.2.3. GLUCOSE TRANSPORTER 1 (GLUT1)

It is the dominant Bovine Mammary Endothelial cells (BMEC) glucose transporter and is expressed at high levels in both the luminal and abluminal membrane.^[31] It is noteworthy that GLUT1 is highly specific for d-glucose transport and only minimal structural changes are allowed for GLUT1 substrate recognition.^[32] Consequently, targeting GLUT1 with chemo-therapeutics conjugated to glucose has been largely unsuccessful.

2.2.4. ORGANIC ANION TRANSPORTING POLYPEPTIDES (Oatps)

It provides intriguing targets for the rational design of CNS drugs as they transport a wide variety of endo- and xeno-biotic substrates.^[33] Endobiotics transported by Oatps include bile acids, conjugated sterols, the opioid peptides enkephalin and deltorphins and one of the most prescribed drugs on the market today, thyroxine. Other drugs transported by Oatps include compounds as structurally diverse as methotrexate, saquinavir, atorvastatin, glyburides and the fenamate class of NSAIDs.^[34]

3. ROUTES FOR DRUG-DELIVERY ACROSS THE BRAIN

- Intranasal route
- Vascular route
- Transcranial route

3.1 INTRANASAL ROUTE

Intranasal administration is a non-invasive method of drug delivery allows therapeutic substances a direct access to CNS. Nasal mucosa has been considered as a potential administration route to achieve faster and higher level of drug absorption as it is more permeable than the gastrointestinal tract and has neutral pH. It is also suitable for drugs degrading in presence of gastric enzymes. Intranasal delivery of large molecular weight biologics such as protein, gene vectors, and stem cells is a potentially useful strategy to treat variety of disease of CNS including stroke, Parkinson's disease, multiple sclerosis, Alzheimer's disease, epilepsy, and psychiatric disorders. It is a useful delivery method for drugs that are active in low doses and show no or minimal oral bioavailability such as proteins and peptides.^[35]

3.1.1 MECHANISM FOR OLFACTORY TRANSPORT OF DRUG TO BRAIN

The major part of the approximately 150 cm² surface in the human nasal cavity is covered by respiratory epithelium, across which systemic drug absorption can be achieved. The olfactory epithelium is situated in the upper posterior part and covers approximately 10 cm² of the human nasal cavity. The nerve cells of the olfactory epithelium provide a direct connection between the brain and the external environment. The olfactory transfer of drugs into the brain is thought to occur by either slow transport inside the olfactory nerve cells to the olfactory bulb or by faster transfer along the perineural space surrounding the olfactory nerve cells into the cerebrospinal fluid surrounding the olfactory bulbs and the brain.^[36]

3.2 VASCULAR ROUTE

In contrast to the inefficiency of craniotomy-based drug delivery to the brain, a trans-vascular route of drug administration, such as intravenous or systemic injection can treat virtually 100% of the neurons in the brain. Every neuron is perfused by its own blood vessel, thus the drug is delivered to the "doorstep" of every neuron in the brain following initial transport across the vascular barrier. The delivery of drugs (or genes) to the brain by the trans-vascular route is so efficient that the drug or gene could be delivered to all parts of the brain once the vascular barrier is traversed. However, in the absence of a BBB drug-targeting system, the trans-vascular route to the brain is virtually impenetrable by the majority of drug candidates.^[37]

3.3 TRANSCRANIAL ROUTE (TCR)

The unique anatomical arrangement of blood vessels and sinuses in the human skull and the brain, the prevalence of a high density of skin appendages in the scalp, extra-

cranial vessels of the scalp communicating with the brain via emissary veins and most importantly, the way that the scalp is used in *Ayurvedic* medical system in treating diseases associated with the brain show that a drug could be transcranially delivered and targeted to the brain through the scalp. Apart from the established routes of drug administration practiced in modern medicine, *Ayurvedic* system of medicine uses a special route which involves oil therapies to the head. These therapies are used for centuries to treat diseases of the central nervous system. TCR describes the passage of an oil solubilized drug moiety across the skin of the scalp including appendages of the skin such as sebaceous glands, walls of the hair follicles and sweat glands, through the cranial bones along with the diploe, the cranial bone sutures, the meninges and specifically through the emissary veins into the brain. The initial vigorous 'rubbing in' of the medicated oil is an essential part of the transcranial drug administration in order to bring the oil into intimate contact with the epithelium of the skin appendages. The TCR is generally considered to be the area of the head lying above the contour drawn through the four points consisting of two corners of the mouth and the two ears in the case of all higher animals.^[38] Systemic side effects of many common drugs could be overcome by the administration through the TCR since the drug targets the brain. In addition, the CNS side effects of drugs administered by other routes could also be overcome by administering the specific antagonists through the TCR.

4. DOSAGE FORMS

Over last few years novel drug delivery systems such as liposomes, micro and nano-emulsions, microspheres, micro and nanoparticles have been used to improve drug permeation to brain.

4.1. LIPOSOMES

Liposomes are non-toxic, biodegradable and biocompatible lipid carrier made up of animal lipid such as phospholipids and sphingolipid. They have advantage of carrying hydrophilic, lipophilic and amphoteric drug molecules entrapped inside or on its micellar surface. Mostly lipids used in liposomal drug delivery are phospholipids which forms self-sustained bilayer structure to form liposomes of various size such as small uni-lamellar vesicles to multi-lamellar vesicles. Brain distribution of long circulating liposomes can be used to directly encapsulate drug molecule to diseased tissues or organs. The basic mechanism by which liposomes achieve brain concentration by crossing blood-brain barrier is by coupling with brain drug transporter vector through absorptive mediated transcytosis.^[39]

4.2. NANOPARTICLES

Nanoparticles are colloidal systems with compact structure where the therapeutic agent is either entrapped within colloidal matrix or coated on the particle surface by conjugation or adsorption. Nanoparticles can provide sustained and controlled drug release. Nanosystems

employed for the development of nano drug delivery systems in the treatment of CNS disorders include polymeric nanoparticles, nano-spheres, nano-suspensions, nano-emulsions, nano-gels, nano-micelles and nano-liposomes, carbon nanotubes, nano-fibers and nano-robots, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and lipid drug conjugates (LDC). The correct mechanism of barrier opening by nanoparticles is not exactly known. But the delivered nanoparticles enter into the brain by crossing the BBB by various endocytotic mechanisms. The polymeric nanoparticles made from albumin or poly (butyl-cyanoacrylate) is reported to enter into the brain by their small size mediated endocytosis. These nanoparticles travel intact and release the drug in brain microenvironment directly which is finally biodegraded due to endocytotic uptake because of very small size by BBB.^[40]

4.3. MICROSPHERES

Microsphere technology is one of the specialized systems becoming popular for designing nasal products, as it provides prolonged contact with the nasal mucosa and thus enhances absorption and bioavailability. In the presence of microspheres, the nasal mucosa is dehydrated due to moisture uptake by it. This results in reversible shrinkage of the cells, providing a temporary physical separation of the tight (intercellular) junctions that increases the absorption of the drugs. Microspheres used in nasal drug delivery is water insoluble but absorb water into matrix resulting swelling of the spheres to form a gel. Some low molecular weight drugs also successfully delivered in microsphere formulation. Microspheres have been reported to be present up to 3-5 hours in the nasal cavity depending upon the bio-adhesivematerial used for formulation. The ideal microsphere particle size requirement for nasal delivery should range from 10 to 50 μm as smaller particles than this will enter the lungs.^[41]

4.4. MICRO-EMULSIONS

Micro-emulsions are clear, stable, isotropic mixtures of oil, water and surfactant, frequently in combination with a co-surfactant. These systems are currently of interest to the pharmaceutical scientist because of their considerable potential to act as drug delivery vehicles by incorporating a wide range of drug molecules. They offer the advantage of spontaneous formation, ease of manufacturing and scale-up, thermodynamic stability, and improved drug solubilization and bioavailability.

Preparing a pharmaceutically acceptable dosage form demands a clear understanding of the micro-emulsion structure, phase behavior, factors leading to its thermodynamic stability, factors influencing drug release from the formulation, requirements of ideal micro-emulsion excipients, and the potential uses and limitations of the micro-emulsion system.^[42]

5. BRAIN DRUG DELIVERY RESEARCH-RECENT ADVANCES

5.1. RECEPTOR-MEDIATED TRANSPORT (RMT)

The BBB expresses RMT systems for the transport of endogenous peptides, such as insulin. The RMT systems operate in parallel with the classical carrier mediated transporters (CMT), which transport certain small molecule nutrients, vitamins and hormones. The RMT systems are portals of entry for large molecule drugs that are attached to endogenous RMT ligands.

5.1.1. MONOCLONAL ANTIBODY (MAb) MOLECULAR TROJAN HORSES (MTH)

Genetic engineering is used to produce either chimeric or humanized forms of the monoclonal antibody.^[43] The most potent antibody-based MTH known to date is monoclonal antibody against the human insulin receptor.^[44] Recently, this antibody has been humanized, and shown to cross the BBB *in-vivo* in non-human primates.^[45] Certain peptido-mimetic MAbs act as ligands for the RMT systems. The peptido-mimetic MAbs acts as MTH to ferry across the BBB an attached drug, protein, anti-sense agent, or non-viral plasmid DNA.^[46] A number of non-antibody delivery systems have been evaluated, including histone, p97, receptor-associated protein (RAP), the tat transduction domain peptide, and other cationic peptides or polymers.^[47] Whereas the transport of ligands such as RAP is hypothesized to be receptor-mediated, the transport of cationic peptides is believed to be mediated by absorptive-mediated endocytosis systems that are based on charge interactions.^[48] Delivery of biopharmaceuticals across the BBB has been reported recently using a related RMT system.^[49, 50] A carrier protein known as CRM197 was used as safe and effective carrier protein in human vaccines and more recently in anti-cancer trials.^[51] CRM197 uses the membrane-bound precursor of heparin-binding epidermal growth factor (HB-EGF) as its transport receptor, which is also known as the diphtheria toxin receptor (DTR). In fact, CRM197 is a non-toxic mutant of diphtheria toxin. Membrane bound HB-EGF is constitutively expressed on various tissues and cells such as blood-brain barrier endothelial cells and several other cells. This means that major sanctuary sites (brain) and cellular reservoirs (T-lymphocytes, monocytes, macrophages) can be reached. Moreover, HB-EGF expression is up-regulated strongly under (inflammatory) disease conditions, which will enhance targeted delivery considerably.^[52] Other applications may relate to other neurotropic infections (e.g. Poliovirus, West Nile virus) or other brain-related diseases (e.g. multiple sclerosis, Parkinson's, Alzheimer's).

5.1.2. TROJAN HORSE LIPOSOMES FOR CNS GENE THERAPY

Gene delivery across the BBB may be ineffective owing to the rapid degradation of extracellular nucleic acids, as well as the pro-inflammatory effects of naked DNA.^[53] Encapsulation of plasmid DNA inside pegylated liposomes eliminates the nuclease sensitivity and pro-

inflammatory effects of the nucleic acid.^[54] Pegylated liposomes are not transported across the BBB.^[55] However, the attachment of a MTH to the tips of the polyethylene glycol strands allows the liposome to engage the BBB RMT system, and this triggers transport of the pegylatedimmuno-liposomes, also called Trojan horse liposomes, across the BBB. The administration of this new technology, to mice, rats, or monkeys is followed 24-48 hrs later by global expression of the non-viral transgene in brain.^[56] The BBB delivery of immuno-liposomes carrying an expression plasmid encoding tyrosine hydroxylase allowed for complete restoration of striatal tyrosine hydroxylase enzyme activity in a model of experimental Parkinson's disease.^[57] The intravenous injection of immuno-liposomes carrying an expression plasmid encoding a short hairpin RNA directed against the human epidermal growth factor led to a 90% increase in survival time of mice with intra-cranial human brain cancer. The pegylatedimmuno-liposome gene transfer technology enables intravenous RNA interference (RNAi) of the brain.

5.1.3. TARGETED NANOPARTICLE BRAIN DRUG DELIVERY SYSTEMS

Nanoparticles are produced from polymeric precursors, and these structures can be formulated to encapsulate a wide variety of pharmaceuticals. The size of nanoparticles is typically 50-200 nm, and such structures are too large to cross the BBB via free diffusion. However, the formulation of nanoparticles with polysorbate-80 has shown to enable BBB transport.^[58] The conjugation of low density lipoprotein (LDL) apo-proteins to the surface of nanoparticles appears to trigger RMT across the BBB via the BBB-LDL receptor.^[59] Recent advances have loaded macrophages with nanoparticle/drug complex *ex-vivo*, followed by the intravenous administration of the cells.^[60] Since activated lymphocytes and/or macrophages cross the BBB, these cells may be used as vehicles for drug delivery to the brain.

5.1.4. IN-VIVO BRAIN IMAGING OF GENE EXPRESSION

Anti-sense radiopharmaceuticals hold promise for imaging gene expression in the brain using nuclear medicine imaging modalities, such as PET or SPECT. However, anti-sense radiopharmaceuticals do not cross the BBB on their own and must be modified if they are to be useful brain gene imaging agents. Peptide nucleic acids can be bio-tinylated and radio-labeled with 111-indium. In parallel, a conjugate or fusion protein, of avidin and a BBB molecular Trojan horse can be synthesized. The peptide nucleic acid is then coupled to the MTH via the avidin-biotin bridge. Such targeted antisense radiopharmaceuticals cross the BBB, and the brain cell membrane, and enable the *in vivo* imaging of gene expression in brain.^[61]

5.2. MECHANISMS TO CIRCUMVENT THE BBB

5.2.1. INTRANASAL DELIVERY

A non-invasive, intranasal method of bypassing the blood-brain barrier to deliver therapeutic agents to the brain has been developed in recent times.^[62, 63] This method allows drugs that do not cross the blood-brain barrier to be delivered to the olfactory cerebrospinal fluid via transport across the olfactory region of the nasal epithelium. The surface area of the olfactory region of the nasal epithelium in rodents is large, about 50%, and is small in humans, about 5%, therefore intranasal delivery is not expected to achieve therapeutic drug levels in most brain regions.^[64]

5.2.2. CONVECTION-ENHANCED DRUG DELIVERY (CED)

CED is a method for local/regional micro-infusion targeted directly to brain tissue and it is used primarily for large molecular weight agents that show minimal leakage across the BBB and/or have significant systemic toxicity, including viruses, oligonucleotides, nanoparticles, liposome and targeted immunotoxins.^[65] Parameters that affect CED volume of distribution include infusion parameters (rate, volume, duration, cannula size), infusate characteristics (molecular weight, surface properties, tissue affinity) and tissue properties (tissue density, extracellular space, vascularity and interstitial fluid pressure).^[66] Animal studies have demonstrated that the volume of distribution achieved by CED can be imaged by magnetic resonance in real time by including contrast agents within the infusate.^[67] The major clinical use of CED will be for targeted therapy of glioblastoma. Recent studies have included interleukin-13/pseudomonas exotoxin alone or in combination with radiation/temozolomide, and radio-immunotherapy with mAbs targeting tenascin or tumor necrosis factor.^[68, 69] Mechanisms for CED treatment failure include distribution inhomogeneity, high interstitial fluid pressure and rapid efflux of agent from the injection site. To overcome these issues, increased residence time must be achieved to enhance targeted toxin receptor binding and uptake by the cancerous cells. Although primarily targeting brain tumors, the CED technique may also gain use for localized neurodegenerative disorders.^[70] Infusion of adenovirus vectors or glial-derived neurotrophic factor has been assessed in Parkinson disease.^[71]

5.2.3. OSMOTIC BBB DISRUPTION (BBBD)

Transient osmotic disruption of the blood-brain, blood-Cerebro-spinal Fluid (CSF), and blood-tumor barriers can be achieved throughout a vascular circulation by intra-arterial infusion of a hyperosmotic agent; usually mannitol.^[72] Osmotic BBBD reversibly opens the BBB by shrinking the cerebrovascular endothelial cells with transient opening of the tight junctions between cells. The BBB is opened to drugs, proteins, and nanoparticles for between 15 minutes (for viral-sized agents) up to 4 hours (for low molecular weight compounds) before

returning to baseline permeability.^[73] BBBD currently is used clinically for the delivery of chemotherapy to the CNS in patients with brain tumors. BBBD increases parenchymal and CSF chemotherapy concentrations by 10-100 folds compared to intravenous administration alone.^[74] Upcoming studies include use of BBBD to improve delivery of radio-immuno-therapeutics in Primary central nervous system lymphoma (PCNSL) and breast cancer metastasis. Osmotic BBBD also has the potential for enhancing delivery of therapeutics to the brain for treatment of brain infection, lysosomal storage disorders, and neurodegenerative diseases.^[75]

5.2.4. BRADYKININ RECEPTOR-MEDIATED BBB OPENING

Bradykinin, an endogenous peptide mediator of the inflammatory response, can induce transient increases in blood vessel permeability that can be highly specific for tumor vasculature. RMP-7 (lobadimil) is a Synthetic bradykinin analog that is specific for the B2 receptor and is 100-fold more potent than bradykinin in mice. Pharmacological manipulation of the BBB offers the possibility of highly specific opening and targeted drug delivery to tumor, albeit with the possibility that increases in delivery may only be modest and dependent on the tumor type or model treated.^[76] However, RMP-7 had no effect on the pharmacokinetics or toxicity of carboplatin, and two studies have shown no objective responses of RMP-7 and carboplatin in brain stem glioma or high-grade glioma.^[77]

5.2.5. ULTRASOUND-MEDIATED BBB OPENING

BBB disruption by MRI-guided focused ultrasound can achieve focal CNS delivery in animal models. Consistent vascular leak without tissue damage can be achieved by localizing cavitation-generated mechanical stresses to blood vessel walls by IV injection of preformed gas bubbles just prior to pulsed ultrasound treatment. Histology showed that the low power ultrasound caused reversible focal opening which was completely healed within 24 hours.^[78] The ultrasound with micro-bubbles exposures did not cause neuronal damage, apoptosis or ischemia, or long term vascular damage. Ultrasound BBB disruption produced clinically relevant levels of liposomal doxorubicin and mAbs in the targeted local areas of the brain in animals.

6. BONE MARROW TARGETED LIPOSOMAL CARRIERS

Liposomes were first described by Bangham in 1960s as self - assembled lipid vesicles composed of one or more lipid bilayers.^[84] They are microscopic closed vesicles consisting of mainly phospholipid bilayers surrounding an aqueous medium.^[85] Phospholipids, when dispersed in an aqueous environment at a concentration above their critical micelle concentration (CMC), tend to form these closed vesicles spontaneously and encapsulate some of the aqueous environment. Most widely used lipids are phospholipids (PLs), especially phosphatidylcholine, phosphatidic acid, phosphatidylglycerol,

phosphatidylserine and phosphatidyl-ethanolamine. PLs have different combinations of fatty acid chains in the hydrophobic region of the molecule with different chain length and degree of unsaturation.^[86] Liposomes vary in size ranging from 30 nm to several micrometers, phospholipid composition, and surface characteristics. These properties can be modified for specific applications. Liposomes composed of single lipid bilayer structures are referred as unilamellar liposomes. Unilamellar liposomes vary in size. Small unilamellar vesicles (SUVs) range in size from 20 to 100 nm whereas liposomes larger than 100 nm are referred as large unilamellar vesicles (LUVs). The diameters of LUVs are in a very broad range; from 100 nm up to cell size and they are called the giant vesicles (GUV).

6.1. UPTAKE OF PARTICLES BY BONE MARROW PHAGOCYTES

The total blood cell production rate in adult human bone marrow is about 4.9×10^{11} cells per day or about 88 kg per year.^[87,88] From the viewpoint of material balance for cell turnover, it is reasonable to speculate that the bone marrow extensively acquires materials from the blood for hematopoiesis. Chylomicrons are large lipoprotein particles that consist of triglycerides, phospholipids, cholesterol, and proteins. Hussain and co-workers have reported that rabbit and marmoset bone marrow had significant uptake of chylomicrons labeled with [^{14}C]cholesterol and [^3H] retinol.^[89,90] Perisinusoidal macrophages protruding through the endothelial cells into the marrow sinuses were responsible for the accumulation of the chylomicrons in the marmoset bone marrow. In contrast to marmosets, chylomicron clearance from the bone marrow of rats, guinea pigs, and dogs was much less, and the spleen in rats and guinea pigs took up a large fraction of chylomicrons. Thus, it was concluded that the observed differences in chylomicron metabolism are due to the presence of perisinusoidal macrophages in bone marrow. It was also believed that the differences between bone marrow and spleen uptake of chylomicrons may provide insights into the role of chylomicron catabolism in these organs, both of which are involved in hematopoiesis. It was speculated that the chylomicrons may have a role in the delivery of lipids to the bone marrow and spleen as a source of energy and for membrane biosynthesis or in the delivery of fat soluble vitamins. In general, it is believed that aged blood cells are cleared by the liver and spleen. However, recent evidence from several sources indicates that the bone marrow is a significant site for clearance of apoptotic neutrophils.^[91,92] The high uptake of white blood cells in bone marrow can also be observed in humans following administration of indium-111 radiolabeled white blood cells that are routinely administered to humans for detection of occult infection. Whole body region-of-interest analysis frequently finds that 60–70% of the administered white blood cells localize to bone marrow while 30–40% localizes to liver and spleen. Interestingly, the uptake of apoptotic neutrophils by bone marrow macrophages has been

shown to stimulate the production of granulocyte-colony stimulating factor. Also, bone marrow macrophages, associated with erythroblasts in a hematopoietic environment, participate in erythropoiesis control, and engulfment of nuclei from erythroid precursor cells.^[93] Recently, Winkler and co-workers reported that the bone marrow macrophages are pivotal to maintain the endosteal hematopoietic stem cell niche and that the loss of such macrophages leads to the egress of hematopoietic stem cells into the blood.^[94] They administered clodronate-loaded liposomes intravenously to deplete the bone marrow macrophages. After depletion of the macrophages, hematopoietic stem cells were found in the blood. These findings provide evidence supporting the critical role that macrophages play in the support of hematopoietic cells in bone marrow. Such specific biology of bone marrow macrophages may offer a therapeutic target for the treatment of hematopoietic disorders. Schettini and co-workers prepared a novel liposomal formulation of meglumineantimoniate, a drug used for treating leishmaniasis, to deliver the drug to the bone marrow.^[95] The liposomes were made from distearoylphosphatidylcholine (DSPC), cholesterol and dicetylphosphate (molar ratio of 5:4:1). The targeting of antimony to the bone marrow was improved (approximately three-fold) with the small liposomal formulation as compared to the large liposome formulation used in dogs with visceral leishmaniasis. These liposomes had no active targeting factor to bone marrow but the passive targeting of the liposomes to the bone marrow of dogs was improved by the reduction of vesicle size from 1200 nm to 400 nm. Recently, other nanoparticles such as guanidinium group-modified nanoparticles and cationic nanoparticles were tested as carriers of oligopeptide and siRNA to deliver the therapeutic agents into bone marrow cells.^[96, 97] Moghimi and co-workers have reviewed the clearance mechanism of particulate materials from the circulation by bone marrow.^[98,99] The endothelium of bone marrow sinusoids is capable of removing particles from the circulation by both transcellular and intercellular routes. The transcellular route occurs through the fenestrate in the endothelial wall; therefore this mechanism is strongly dependent on particle size. The size of the fenestrate was reported to range from 85–150 nm.^[100] Liposomes consisting of DSPC, cholesterol, PEG (5000)-DSPE and α -tocopherol and prepared in various sizes (136–318 nm in diameter) have been tested for organ distribution in rabbits, and none of these liposomes show a significant accumulation in bone marrow.^[101] Other possible mechanisms of liposome recognition by phagocytic cells may occur via binding plasma proteins. Several types of proteins such as immunoglobulins, complement proteins, apolipoproteins, fetuin, von Willebrand factor, and thrombospondin have been identified as ligands for the macrophage, and these binding proteins are known to accelerate uptake by hepatic Kupffer cells.^[102] Hematopoietic factors such as erythropoietin and iron transporting transferrin have been suggested as possible

serum proteins that could provide specificity for bone marrow.

7. RECENT WORKS ON BONE MARROW DRUG DELIVERY

Regarding engineered colloidal particles, Porter and co-workers observed remarkable accumulation of small colloidal particulates (150 nm and smaller diameter) that were coated by the block co-polymer poloxamer-407, a non-ionic surfactant, in the bone marrow after intravenous administration in rabbits.^[103] In this case, the coated colloids were sequestered by the sinusoidal endothelial cells of the bone marrow instead of macrophages. Importantly, no marked uptake was achievable with other block co-polymers having a similar structure to that of poloxamer-407, suggesting the participation of a specific interaction mechanism between the particle and the sinusoidal endothelial cell surface. Chi and co-workers prepared dendritic amine and guanidinium group-modified nanoparticles for the delivery of model peptide drug into primary osteoclast precursor cells (bone marrow macrophages).^[104] It can be speculated that positively charged guanidinium groups have favorable interactions with negatively charged functional groups in the cell membrane of osteoclast precursors.^[105] Physicochemical interaction between a positively charged drug carrier and a negatively charged cell surface can enhance the cellular uptake in cells of various kinds with a negatively charged surface. Harris and co-workers studied tissue-specific gene delivery via nanoparticle coating.^[106] They prepared cationic nanoparticles with plasmid DNA and then coated their cationic surface with poly anionic poly(glutamic acid)-based peptides with and without cationic insert. Particles coated with a low 2.5:1 peptide: DNA weight ratio (w/w) form two large micro-sized particles that can facilitate specific gene delivery to the liver in mice. However, the same particles coated at a higher 20:1 peptide with cationic insert: DNA (w/w) form small 200 nm particles that can facilitate specific gene delivery to the spleen and bone marrow. They have confirmed that the terminal sequence insert, cationic amino acid sequence G-dP-dL-G-dV-dR-G, to the poly(glutamic acid)-based peptides is a critical factor enhancing bone marrow and spleen-specificity of gene delivery in vivo. Regions of luminescence selected around the femur bones showed nearly 40-fold enhancement, and regions around the spleen showed nearly 30-fold enhancement by the cationic insert. Flow cytometry analysis of bone marrow cells from a mouse tail-vein injected with green fluorescent protein-encoding nanoparticles coated with 20:1 w/w peptide with a cationic insert revealed that green fluorescent protein expression relative to the whole population of bone marrow cells is enriched in monocyte and T-cell lineage cells. This system might be available for bone marrow-specific drug delivery and for gene delivery. E-selectin is an attractive molecular target for active targeting of a drug carrier to bone marrow because E-selectin is expressed selectively on endothelial cells of adult and fetal hematopoietic organs.^[107] It has been

suggested that the E-selectin plays a role in the homing of hematopoietic progenitor cells and that its constitutive expression on endothelial cells of hematopoietic organs is necessary in the initial step of the homing process. Mann and co-workers identified a thiophosphate-modified aptamer (thioaptamer) against E-selectin. They confirmed that the thioaptamer ligand selectively binds to E-selectin with nanomolar binding affinity ($K_D=47$ nM) while exhibiting minimal cross reactivity to P-selectin and L-selectin. Recently, they developed porous silicon particles modified with E-selectin thioaptamer ligands to target bone marrow endothelium.^[108] A mice study demonstrated that the accumulation of the porous silicon particles modified with E-selectin thioaptamer ligands in the bone marrow was eight times higher than control porous silicon particles, which were accumulated primarily in the liver and spleen instead of bone marrow. Histological analysis supported the presence of porous silicon particles modified with E-selectin thioaptamer ligands on the endothelial wall of the bone marrow tissue. The molecular target of this ligand-receptor reaction might be readily apparent in this system and be promising for delivering drugs to bone marrow endothelial cells specifically.

8. FUTURE PROSPECTS FOR BONE-MARROW-TARGETED DRUG CARRIER SYSTEMS

The balance between blood cell production and removal in blood cells of each type is important to maintain the number of circulating blood cells. When blood cell production is suppressed, erythrocytopenia, leukopenia, and thrombocytopenia are induced. The blood cell production suppression results from the direct bone marrow dysfunction or indirect factor such as deficiency of the hematopoietic growth factors such as erythropoietin (EPO), granulocyte colony-stimulation factor (G-CSF) and thrombopoietin (TPO). These hematopoietic growth factors, EPO and G-CSF, have been established as recombinant products. Recombinant EPO products are used clinically for the renal anemia patients or autologous blood. These growth factors function with hematopoietic cells in bone marrow, so that a drug delivery system to target bone marrow can be expected to offer an improved therapeutic system. Recently, Winkler and co-workers reported that the bone marrow macrophages are pivotal to maintain an endosteal hematopoietic stem cell niche and that the loss of such macrophages endangers the progress of hematopoietic stem cells into the blood.^[109] They administered clodronate-loaded liposomes intravenously to deplete the bone marrow macrophages. After the macrophage depletion, hematopoietic stem cells were found in the blood. These findings provide evidence supporting the critical role that macrophages play in the support of hematopoietic cells in bone marrow. Such specific biology of bone marrow macrophages can present a therapeutic target for the treatment of hematopoietic disorders. Leishmaniasis is an infectious disease caused by a protozoan parasite that is parasitic on

resident macrophages. The typical symptoms in leishmaniasis are damage to the spleen and liver, and anemia by damage to bone marrow. Promastigotes, which are injected into the skin by a sandfly, are phagocytized by macrophages. Thereafter, promastigotes transform into amastigotes inside macrophages. The amastigotes multiply in cells, including in macrophages, at various tissues. Therefore, macrophages in bone marrow as well as in the liver and spleen are target cells in leishmaniasis treatment. Thus several nanoparticles loaded with therapeutic agents such as nanoparticles loaded with amphotericin B^[110, 111], PLGA nanoparticles loaded with saponin, lipid nanoparticles loaded with oryzalin, PLGA nanoparticles loaded with kinetoplastid membrane protein-11 (Santos *et al.*, 2012)^[112] have been developed to deliver the drugs in leishmania-infected macrophages. Drug carriers targeting bone marrow,

especially bone marrow macrophages, would have a great potential to deliver these therapeutic agents in leishmania-infected macrophages. The abnormal increases of cancerous cells such as leukemia cells, which are immature white blood cells in bone marrow, suppress normal hematopoiesis. Different from a solid cancer, surgical resection is ineffective as a treatment for leukemia. Most cases of leukemia are treated using chemotherapy. Therapies are typically combined into multi-drug chemotherapy. Drug delivery systems targeting bone marrow can increase the efficacy of such methods. Moreover, bone marrow is sensitive to chemotherapy and radiation therapy. Several pharmaceuticals are available to protect soft tissues from chemotherapy and irradiation. Drug carriers targeting bone marrow offer a promising platform to deliver such pharmaceuticals to bone marrow effectively.

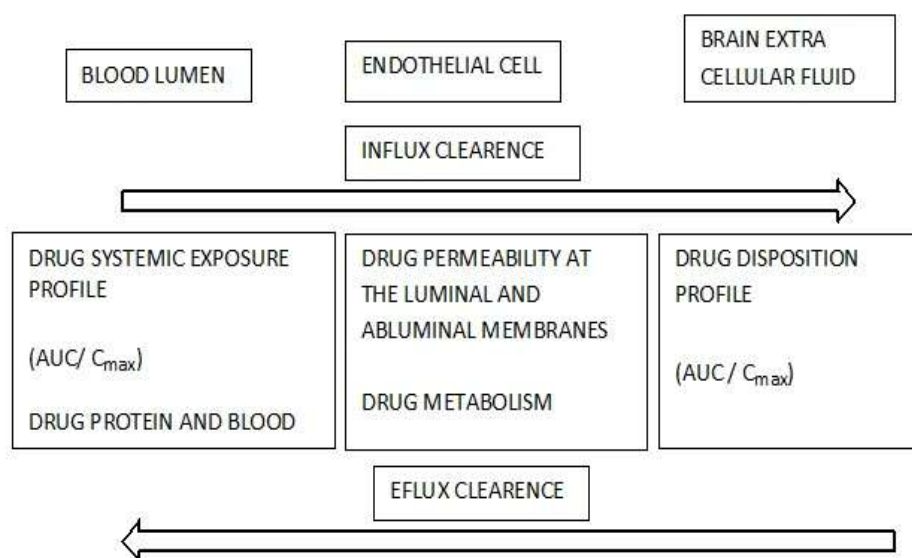


Fig. 1:- The net uptake of a drug by the brain through the endothelial cell forming the blood–brain barrier depends on the overall difference between the uptake and efflux processes. AUC=area under the blood–time curve and C_{max} = maximal blood concentration.

9. CONCLUSION

After carrying out the review work on drug delivery to brain, a number of drug delivery systems came to light such as drug delivery by – Liposomes, Nanoparticles, Microsphere, Microemulsions, etc. In addition to enhanced brain transport, these systems also provide additional advantages such as extended or controlled release of drugs and protection from degradation before reaching the targeted site leading to decreased dose or lesser frequency with decreased or no side effects. Numerous drug molecules have been made to be permeated through blood brain barrier by incorporating in suitable nanoformulations which resulted in enhanced efficacy of the drug and better therapeutic action. Further, drug delivery through the Intra-nasal route claims to be a potential route for drug delivery to brain. The current challenge is to develop drug delivery systems that ensure the safe and effective passage of

drugs across the BBB. So, further studies and research on these drug delivery systems and routes can prove to be fruitful. Bone marrow proves to be a challenging target for drug delivery. Surface-modified liposomes provide a promising methodology to deliver liposomal drugs into bone marrow via specific bone marrow phagocytosis. Potential clinical uses of bone marrow delivery system include the delivery of agents that protect the marrow from the toxic effects of chemotherapy and radiation, and the delivery of agents to effectively and safely ablate bone marrow prior to bone marrow transplant. The therapeutic systems can target bone and bone marrow diseases such as rheumatoid arthritis, bone regeneration and repair, bone metastases, osteoporosis, infectious diseases, multiple myeloma and hematopoietic dysfunction. Also, the ability to specifically deliver stimulants of hematopoietic cell proliferation may open novel therapeutic system for increasing hematopoiesis.

Development of an effective bone marrow delivery system in humans could prove valuable due to the vital importance of the bone marrow as a crucial hematopoiesis site.

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