CHAPTER - 1

Targeted Drug Delivery Systems

Concepts, Biological Process Involved in Drug Targeting, Tumour Targeting and Brain Specific Delivery

CONCEPTS

About a century before Ehrlich introduced the concept of drug targeting to a specific site in the body[1]. Only in recent years the field has emerged as an important area of research. During the twentieth century this long silence in the field was attributed to an inadequate understanding of various diseases; at the cellular-molecular level, a lack of detailed description of how the drugs are processed; and difficulties in identifying and producing carrier molecules specific to the targeted organs, cells, or tissues. The recent advent of Mu and progress in biochemical pharmacology and molecular biology have not only provided a clearer explanation of pathogenesis of many diseases and identification of various types of surface cell receptors. The production of several new classes of highly potent protein and peptide drugs for example, homo- and hetero-logous peptidergic mediators and sequence-specific oil-gonucleotides[2] has been enabled. For the new drugs, and for some traditional drugs such as antineoplastic agents that have narrow therapeutic windows and require localization to a particular site in the body. It is essential that these drugs are delivered intact to their target sites in required concentrations, and in a safe, efficient, convenient, and cost-effective way. Currently most of the drug therapies available provide little, if any, target specificity. The selective delivery of drugs to their pharmacological receptors should not only increase the therapeutic effectiveness, but also limit side effects and increase safety. Many of the drugs that have been included in target-specific delivery systems and the therapeutic impact of this technology on disease state management are comprehensively treated, with the goal of providing an insight into the rapid developments.

After administration of a traditional immediate- or controlled-release dosage form, most drugs freely distribute throughout the body. This leads to uptake by the cells, tissues, or organs where their pharmacological receptors are present. The Figure 1.1 illustrate the distribution, metabolism, and elimination of drugs occur after absorption by natural pathways. The Figure

also demonstrate no specific target for most part of the body. For example, drugs administered through oral route must withstand large fluctuations in pH when it passes through the gastrointestinal (GI) tract and resist the onslaught enzyme attack that digest the food and metabolism by microflora that live there. A systemically active drugs are absorbed from the GI tract into the blood before it crosses the region of absorption in the tract. Once in the blood, it is required to survive inactivation by metabolism and extraction (first-pass metabolism). Therapeutic effect of the drug can then be exhibited when the drug access and interact selectively with its pharmacological receptors. At the active site, there must be adequate concentration of the drug. However, administration of drug through parenteral route avoids GI-associated problem, but deactivation and metabolism of the drug and dose related toxicity are frequently observed. Moreover, it cannot be assured that, among all the paths the drug after administration may take one of these so that the adequate concentration of drug can reach to its desired destination. There are many diseases for which accessibility of the drug is less, for example, rheumatoid arthritis, diseases of the central nervous system, some cancers, and intractable bacterial, fungal, and parasitic infections. The treatment of these diseases often requires high doses and frequent administration of drugs, which can lead to toxic manifestations, inappropriate pharmaco-disposition, untoward metabolism, and other deleterious effects. Reasons enumerating why it is preferable to direct drugs to their sites of action are listed in Table 1.1[3]. Thus, drugs (target-oriented drug-delivery system) can be supplied selectively to its site of action in a way that gives maximum therapeutic activity. This happens through controlled and predetermined drug-release kinetics, preventing degradation or inactivation during transit to the target sites, and protecting the body from adverse reactions because of inappropriate disposition. For drugs having a low therapeutic index (ratio of toxic dose to therapeutic dose), targeted drug delivery may provide an effective treatment at a relatively low drug concentration. For target-oriented drug delivery, other requirements include that

(a) the delivery system should be biochemically inert (non-toxic), nonimmunogenic, and physiochemically, *in vivo* and *in vitro* stable;

(b) the carrier must be biodegradable, or readily eliminated without any problem; and

(c) the preparation of the delivery system must be reproducible, cost-effective, and reasonably simple.

Table 1.1 Reasons for site-specific delivery system.

Parameter	Reasons for site-specific delivery system
Pharmaceutical	• Instability of drugs when delivered from conventional dosage form • Solubility
Biopharmaceutical	• Low absorption • High membrane binding • Biological instability
Pharmacokinetic & Pharmacodynamic	• Short half-life • Large volume of distribution • Low specificity
Clinical	• Low therapeutic index • Anatomical or cellular barriers
Commercial	• Drug presentation

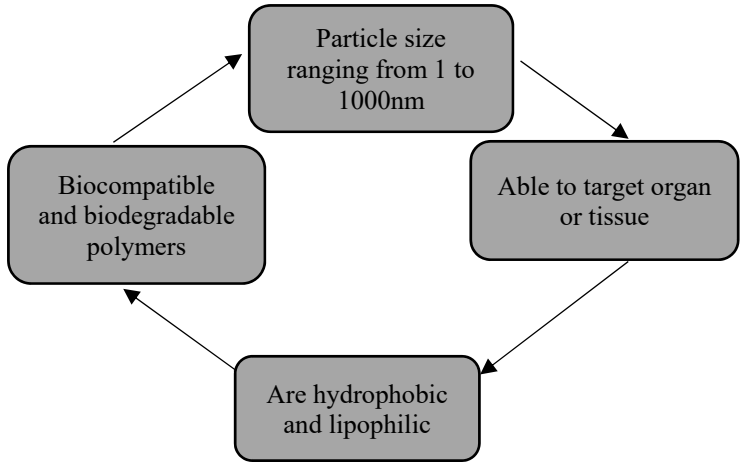

Fig.1.0 Nanoparticles used as a targeting drug delivery system.

BIOLOGICAL PROCESSES INVOLVED IN DRUG TARGETING

Drug targeting has been classified into three categories:

(1) First-order targeting - This describes the delivery of a drug to a discrete organ or tissue;

(2) Second-order targeting - This represents targeting to a specific cell type within a tissue or organ, for example, tumour cells versus normal cells and hepatocytic cells versus Kupffer cells; and

(3) Third-order targeting - This implies delivery to a specific intracellular compartment in the target cells, for example, lysosomes[4].

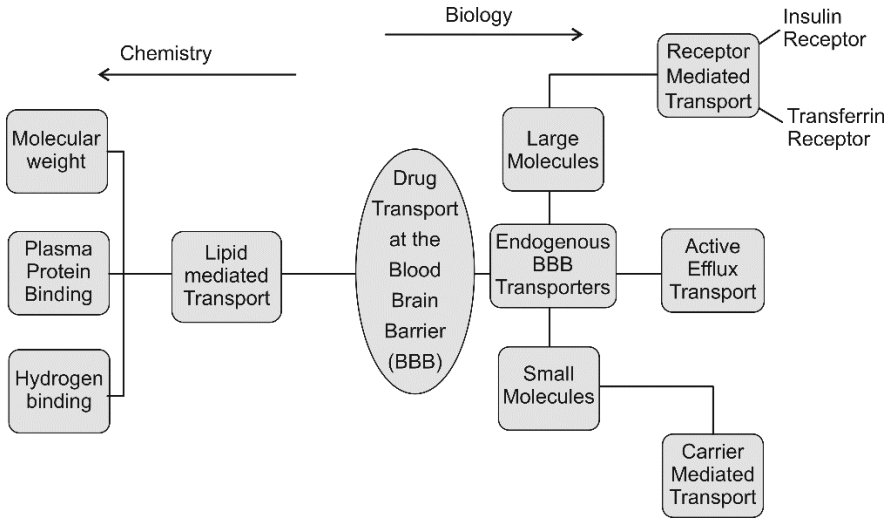

Fig.1.1 Outline of a development program of blood-brain drug targeting strategies.

Primarily, for drug targeting there are three methods:

(a) This method involves the use of biologically active agents that are both potent and selective to a particular site in the body (magic bullet approach of Ehrlich).

(b) This approach involves the preparation of pharmacologically inert forms of active drugs, which after reaching the active sites become activated by a chemical or enzymatic reaction (prodrug approach).

(c) The third method utilizes a biologically inert macromolecular carrier system that directs a drug to a specific site in the body where it is accumulated and effects its response (magic gun or missile approach).

Irrespective of the type of approach/ method the therapeutic efficacy of targeted drug delivery systems depends on the timely availability of the drug in active form at the target site(s) and its intrinsic pharmacological activity. The intrinsic pharmacokinetic properties of the free drug should be same, irrespective of whether or not it is introduced into the body attached to a carrier. Figure 1.2 shows the schematic representation of possible anatomical and physiological pathways that a drug may follow to reach its target site(s)[5]. As shown in the figure, a drug can selectively access to, and interact with, its pharmacological receptors, either passively or by active processes. Passive processes depend on the normal distribution pattern of a drug-drug carrier system, whereas the active processes use cell receptor- recognizing ligand(s) or antibodies (homing or vector devices) to access specific cells, tissues, or organ in the body. Various biological processes and events regulate drug targeting.

- **Cellular Uptake and Processing**

After administration, a drug frequently crosses the various cells, membranes, and organs to reach its target site(s). Various passive or active processes or mechanisms by which the drug can achieve this state[6]. These mechanisms give the scope for selection of cells and access by targeted drug delivery. Low molecular weight drugs can enter into, or pass through, various cells by simple diffusion processes. Often targeted drug-delivery systems comprise macromolecular assemblies; as a result, these assemblies cannot enter into the cells by such simple diffusion processes. Often targeted drug-delivery system comprise macromolecular assemblies and are unable to enter into cells by such simple processes. Instead, they are captured by a process called endocytosis. Endocytosis is defined as a phenomenon that involves internalization of the plasma membrane, with concomitant engulfment of the extracellular material (fluid or particulate). This process can be constitutive or non-constitutive. Other methods of gaining access to cells include passive diffusion, membrane fusion, and binding to specific or nonspecific regions of the cells. Endocytosis is divided into two types:

- Phagocytosis, and
- Pinocytosis.

Phagocytosis refers to the capture of particulate matter, whereas the latter represents engulfment of fluid. It is carried out by specialized cells of the mononuclear phagocytes system (MPS). Phagocyte is mediated by the absorption of specific blood components such as immunoglobin (Ig) G, component C3b, and fibronectin, called opsonins, and relevant receptors located on macrophages. The extent to which a drug is opsonized, and by what plasma protein, depends on the size and surface characteristics of the particles. This, in turn, determines the engulfment mechanism. For. example, red blood cells treated with glutaraldehyde are opsonized by IgG and quickly phagocytosed by the Fe receptor. On the other hand, cells treated with n-ethylmaleimide are opsonized C3b factor and are engulfed with. a minimal membrane-receptor contact. The patients having changes in the levels of glycoprotein may result in variations in opsonization of administered particles and, consequently, their ultimate distribution in the body[7]. Particles with higher hydrophilic surface characteristics tend to undergo opsonization to a lesser extent and, as a result, show decreased phagocytic uptake[8-11]. This has direct implications in targeting microparticulate drugs to cells other than those of the reticuloendothelial system (RES), because if the drug stays for a longer period in the central circulation, the chances of uptake by other cells would be greater. Nonspecific phagocytic uptake of particles, triggered by particle size and hydrophilic coatings[12] and mediated by membrane components[13], has also been reported.

After ingestion, the phagocytic vacuole or phagosome fuses with one or more lysosomes to form phagolysosome or secondary lysosomes. Thus, digestion of particles by lysosomal acid hydrolases for example, proteinases, glycosidases, nucleases, phospholipases, phosphatases, and sulphatases occurs, leaving the drug available to exert its therapeutic effect. The internal pH of lysosomes is 4.5 and 5.5.

Compared with phagocytosis, pinocytosis appears to be a universal phenomenon in all cells, including phagocytes. Pinocytosis does not require any external stimulus. Pinocytosis is divided into two types: fluid-phase pinocytosis and adsorptive pinocytosis. Fluid-phase pinocytosis is a nonspecific, continuous process, and it is believed to be useful as a general process for transporting macromolecular concentrates through epithelia, some endothelia, and into various blood cells. Adsorptive pinocytosis refers to internalization of macromolecules that bind to the cell surface membrane. If the macromolecule adheres to a general cell surface site, then uptake is referred to as simply nonspecific pinocytosis. If it binds to a specific cell receptor site, the process is called receptor-mediated pinocytosis. Before membrane internalization, the pinocytic substrate frequently patches into domains or areas of the membrane called coated pits. Coated pits have a cytoplasmic coat consisting of clathrin and other proteins. Once internalized, the pinocytic vesicles can interact among themselves or with vesicles of other intracellular origins, such as endosomes and lysosomes. Endosomes are rich in pinocytic receptors. They contain an active ATP-powered proton pump which is not same with that present in in lysosomes. This maintains the internal pH between 5.0 and 5.5. The mild internal acidic pH condition induces dissociation of receptor-drug carrier complex, making the receptor free for recycling. Endosomes also act as a sorting station to route internalized substrates to their appropriate intracellular locations. Internalized substrates that remain intact Internalized substrates that remain induct in the endosome are usually then transferred to the lysosome (transcellular transfer), where their digestion by acid hydrolases continues. In some cells (leg, endothelial cells) the endosomes, instead of transferring their contents to the lysosome, release them outside the cell. This process is termed diacytosis or retro endocytosis and can achieve a vectorial translocation of substances through an otherwise impenetrable barrier of cells[14]. In cells such as secretory polymeric IgG in the neonatal gut, polymeric (f4 in hepatocytes, and low-density lipoprotein (LDL) in endothelia, the secondary lysosome transports its contents to the other side of the membrane by a process called *transcytosis*. The secondary lysosome can also regress to form residual bodies that continue to retain non- degraded macromolecules.

Nonspecific pinocytic uptake appears to be depending on (Hit on the size, molecular weight and configuration), charge, and hydrophobicity of the pinocytic substrates. Polycation macromolecules have increased pinocytic

uptake in rat yolk sacs and rat peritoneal macrophages cultured in vitro, compared to neutral and anionic macromolecules[15-17]. The rate of pinocytic uptake in different cells also increases with an increase in the size and hydrophobicity of the substrates. The molecular size of the pinocytic substrate is detrimental to the movement of macromolecules from one compartment to another.

The receptor-mediated form of endocytic uptake has been identified for a wide variety of physiological ligands, such as metabolites, hormones, immunoglobulins, and pathogens (e.g., virus and bacterial and plant toxins). Some endosomotropic receptors in cells are shown in Table 1.2.

Table 1.2 Distributions of some endosomotropic receptors (Different species).

Cell	Receptor for
Hepatocytes	Galactose, low. density lipoprotein, polymeric IgA
Macrophages	Galactose (particles), mannose-fucose, acetylated LDL, α_2-macroglobulin-protease complex (AMPC)
Leukocytes	Chemotactic peptide, complement C3b, IgA
Basophils, mast cells	IgA
Cardiac, lung, diaphragm endothelia	Albumin
Fibroblasts	Transferrin, epidermal growth factor, LDL, mannose-6-phosphate, Transcobalamin II, AMPC, mannose
Mammary acinar	Growth factor
Enterocytes	Maternal IgG, dimeric IgA, transcobalamin-B_{12}/ intrinsic factor
Blood-brain endothelia	Transferrin, insulin

Compared with phagocytosis, fluid-phase pinocytic capture of molecules is relatively slower, being directly proportional to the concentration of macromolecules in the extracellular aid. It is also dependent on the size (molecular weight) of macromolecules; lower molecular weight fractions are captured faster than the higher molecular weight fractions. The magnitude of the rate of capture by adsorptive pinocytosis is higher than fluid-phase pinocytosis and relates to the nature of substrate-membrane interactions.

- **Transport Across the Epithelial Barrier**

The initial steps in the analysis of net transport across any epithelium are to define the thermodynamic properties of the two surrounding solutions (e.g., concentrations, or preferably activities, of solutes; electrical potential; pressure; etc.) and to measure the transepithelial flows of solutes and solvent. Then, viewing the multicellular structure (including unstirred, extraepithelial layers) as a homogeneous barrier, an attempt is made to define the forces

responsible for a given flow. The theory of nonequilibrium (irreversible) thermodynamics is ideally suited for this purpose. This approach is entirely phenomenologic and does not depend upon a detailed (and often lacking) understanding of structure and function. Consequently, it can provide only limited insight into underlying mechanisms of transport. Nevertheless, by identifying the generalized forces responsible for a given flow, it provides direction for further efforts aimed at defining transport mechanisms at the molecular level in terms of membrane structure and biochemistry. The purpose of this communication is to illustrate briefly the application of nonequilibrium thermo- dynamics to the analysis of flows of solute and solvent across epithelial tissues. The emphasis will be on the analysis of solute movements because it is widely accepted that, in the absence of transepithelial hydrostatic pressure differences, solvent (water) flow is dependent upon solute flow and transepithelial solute concentration differences[18-22].

The buccal, oral, nasal, vaginal, and rectal cavities are all internally lined with one or more layers of epithelial cells. Depending on the position and function in the body, epithelial cells can be of varied forms, ranging from simple columnar, to cuboidal, to squamous types. Irrespective of their morphological differences, these cells are extremely cohesive. The lateral membrane of these cells exhibits several specialized features that form intercellular junctions (tight junction, zonula adherents, and gap junction), which serve not only as sites for adhesion, but also as seals to prevent flow of materials through the intercellular spaces (paracellular pathway) and to provide a mechanism for intercellular communication. The strong intercellular cohesion is partly due to the binding action of the glycoproteins, which are an integral part of the plasma membrane and of a small amount of the intercellular proteoglycan. Calcium ions also play a role in maintaining this cohesion. Below the epithelial cells is a layer of connective tissue called the lamina propria, which is bound to epithelium by the basal lamina. The latter also connects epithelium to other neighbouring structures. The laminal side of the epithelium is covered with a more or less coherent, sticky layer of mucosa. This layer first interacts with foreign materials such as food, drugs, bacteria and chemicals. Mucous contains the glycoproteins (mucins), water, electrolytes, sloughed epithelial cells, enzymes, bacteria and bacterial products, and various other materials, depending on the source and location of the mucus. Mucin is synthesized by goblet cells or by special exocrine cells, acini, and constitutes about 5% of the total weight of the mucus. Structurally mucin is composed of polypeptide with oligosaccharide side chains. Each oligosaccharide contains 8 – 10 monosaccharide residues of a molecular weight of 320 – 4500 and has sialic acid or L-fucose as the terminal group. The oligosaccharide side chains are covalently linked to hydroxyamino acids, serine, and threonine residues along the polypeptide backbone.

The absorption of low molecular weight drugs from the buccal, oral, nasal, vaginal, and rectal cavities is well established. Various transport processes are used by drugs to cross the epithelial barrier lining. These cavities include passive diffusion, carrier-mediated transfer systems, selective and nonselective endocytosis. Moreover, polar materials can diffuse through the tight junctions of epithelial cells (paracellular route).

According to Harris[23] the nasal administration of biopharmaceuticals (polypeptides) caused 1 – 20% of bioavailability of the administered dose, depending on the molecular weight and physicochemical properties of the drug. It is widely accepted that macromolecules having molecular weight less than 10 000 can be absorbed from the nasal epithelium into the systemic circulation in sufficient amounts without the requirement for added materials, except for bioadhesives[24]. Larger molecules, such as proteins for example, interferon, granulocyte colony-stimulating factor (G-CSF), human growth hormone, etc. require both a penetration enhancer (for example, bile salts and surfactants) and bioadhesives. Since the entire dose crosses one tissue, these flux enhancers may cause damage to the nasal mucosa and mucillary function. Thus, preventive measures must be taken in using them. Recently, cyclodextrin[24] and phospholipids[25] have been reported to significantly increase the absorption of macromolecules, without causing any damage to the nasal mucosal membrane. The phospholipid approach is attractive, in that phospholipids are biocompatible and bioresorbable and, thus, pose no threat of toxicity.

The transport of macromolecules across intestinal epithelium may occur by cellular vesicular processes. This involves either fluid-phase pinocytosis or specialized processes[26,27]. The spheres of 20 nm diameter, if administered orally to suckling mice, these can pass through the epithelial layer and localized in the Kupffer cells of the lumen, the mesenteric lymph nodes, and even the thymic cortex. With poly alkyl cyanoacrylate nanocapsules smaller than 300 nm in size have been studied recently. The report of this study suggest that particles can pass through the intact intestinal barrier by the paracellular route[28]. In Peyer's patches containing M cells have been suggested to transport particles existing within the epithelium membrane. These cells are known as specialized absorptive cells, these can absorb and transport indigenous bacteria such as *Vibrio cholera*; macromolecules, such as ferritin ad horseradish peroxidase; viruses; and carbon particles, from the lumen of the intestine to submucosal lymphoid tissue[29]. Moreover, transport of absorbed materials to the systemic circulation through lymph fluid and by lymphocytes has been suggested as possible. An increase in lymph flow or a decrease in the blood supply could make lymphatic uptake of particle imprtant[30]. Since Peyer's patches are more prevalent and larger in young individuals and drastically decrease with increasing age, the transport by this route may only be of significance in younger individuals[31].

Various factor influencing the absorption of drugs, including peptides, from the GI tract have recently been reviewed[32,33]. Table 1.3 shows some of the parameters such as pH, enzymes, surface area, segment length, microflora, and transit time. A variety of penetration enhancers have been used to improve intestinal absorption of peptides and other macromolecular drugs. These are chelators such as ethylenediaminetetraacetic acid, citric acid, salicylates, N-acetyl derivatives of collagen, and enamines; natural, semisynthetic, and synthetic surfactants for example, bile salts, derivatives of fusidic acid, sodium lauryl sulphate, polyoxyethylene-9-laurylether, and polyoxyethylene-20-cetylether; fatty acids and their derivatives for example, sodium caprate, sodium laurate, oleic acid, monoolein, and acrylcarntines; and a variety of mixed micelle solution[34,35]. The different regions of the GI tract show different sensitivity to penetration enhancers. The different order of sensitivity of different regions of GI tract can be shown as:

rectum > colon > small intestine > stomach. It is shown that administration of peptides and proteins results in less than 1% bioavailability[36]. There is very little proof that soluble or particulate macromolecules can be transported across the buccal mucosa[37]. However, more work is required to determine whether this route could be of any use in drug targeting.

The absorption of the drugs from the rectal cavity[38] has been extensively studied. This has been found that the absorption and lymphatic uptake of soluble and colloidal macromolecules can be increased significantly, if the rectal mucosal membrane with lipid-nonionic surfactant mixed micelles are pre-treated. There was no evidence of serious damage of the mucosal membrane. It has been suggested that the vaginal cavity could be an effective delivery site for some pharmaceuticals, such as calcitonin, for the treatment of postmenopausal osteoporosis.

- **Extravasation**

From the dysfunction of cells outside the cardiovascular system, many diseases may occur. For a drug to exert its therapeutic effect. For exerting therapeutic effect, a drug must egress from the central circulation and interact with its extravascular-extracellular or extravascular-intracellular targets. This process of transvascular exchange is called extravasation. It is governed by the permeability of blood capillary walls. The biological features mainly control the permeability of capillaries include the structure of the capillary wall, under normal and (patho)physiological conditions, and the rate of blood and lymph supply. The important physicochemical factors of compounds in extravasation are molecular size, shape, charge, and hydrophilic-lipophilic balance (HLB) characteristics.

Table 1.3 Factors influencing the absorption of drugs.

Location	Average Length/dia. (cm)	Average Surface area (m^2)	Average pH (range)	Enzymes & others	Mean Transit time	Microflora Per gram content
Mouth cavity	15 – 20//10	0.07	6.4 (5.8–7.1)	Ptyalin, Maltose, Mucin	9 – 15s	
Esophagus	25/2.5	0.02	5.6		0.5 – 4.5h	
Stomach	20/15	0.11	1.5 (1.0 – 3.5)	Pepsin, Lipase, Rennin, HCl		
Duodenum	25/5	0.09	6.9 (6.5 – 7.6)	Bile, Trypsin, Chymotrypsin, Amylase, Maltase, Lipase, Nuclease, Peptidases		$< 10^3$
Jejunum	300/5	60	6.9 (6.3 – 7.3)	Erepsin, Amylase, Maltase, Sucrase, Peptidases	1 – 4 h	
	300/5	60	7.6 (6.9 – 7.9)	Lipase, Nuclease, Enterokinase, Nucleotidase, Peptidases		$10^5 – 10^7$
	10 – 30/7	0.05	7.7 (7.5 – 8.0)		4 – 16 h	
Colon	150/5	0.25	7.95 (7.9 – 8.0)			$10^{10} – 10^{13}$
Rectum	15 – 19/2.5	0.015	7.7 (7.5 – 8.0)		2 – 8 h	

The structure of the blood capillary wall is complex. In different organs and tissues it varies. It consists of a single layer of endothelial cells joined together by intercellular junctions. On an average, the length of each endothelial cell is within 20 – 40 μm, width is within 10 – 15 μm and 0.1 – 0.5 μm thick. About 10,000 – 15,000 uniform, spherical vesicles called plasmalemmal vesicles are contained by each endothelial cell. The diameter of these vesicles range from 60 to 80 nm. Approximately 70% of these vesicles remain open on the luminal side of the endothelial surface, and the remaining 30% of the vesicles remain open within the cytoplasm. It is believed that plasmalemmal vesicles are involved in the pinocytic transport of substances throughout the endothelium. The pinocytic vesicles take about one second for their transition across the cell. Plasmalemmal vesicles fuse together and form trans-endothelial channels. On the luminal side, the endothelial cells are covered with a 10 – 20 nm thick layer of a glycosaminoglycan coating. This layer continues into plasmalemmal vesicles and into trans-endothelial channels. It is thought that this layer adheres the cells, stabilizes the receptors, protects the cells, and regulates extravasation. It also provides many microdomains of differing charge or charge density on the endothelial cell surface. On the external side, the endothelium is supported by a 5 to 8 nm-thick membrane called the basal lamina. Below the basal lamina there is a layer of connective tissues, it is called *adventitia*. The basal layer is surrounded by connective tissues and blending externally with the surrounding fibroaerolar tissues.

Depending on the morphology and continuity of the endothelial layer and the basement membrane, blood capillaries are divided into three types:

1) Continuous,
2) Fenestrated, and
3) Sinusoidal.

The distribution of these capillaries in the body and their characteristics are shown in Table 1.4.

Continuous capillaries are common and widely distributed in the body. They exhibit tight inter-endothelium junctions and an uninterrupted basement membrane. Fenestrated capillaries show inter-endothelium gaps of 20 – 80 nm at irregular intervals. These gaps have a thin membrane. This is thought that this membrane is derived from the basal membrane. Sinusoidal capillaries show inter-endothelial gaps up to 150 nm. Depending on the tissue or organ, the basal membrane in sinusoidal capillaries is either absent, for example, in liver, or present as a discontinuous membrane, for example, in spleen, and bone marrow. Sinusoidal capillaries are also wider in diameter, have an irregular lumen, and their wall is very thin. Moreover, they have hardly any connecting tissues between the endothelial cells and the cells in which they

are present. This area is occupied by a variety of cells, including highly active phagocytic cells.

Table 1.4 Distribution and characteristics of endothelium in different tissues.

Tissue	Characteristics
Continuous Endothelium Connective tissue, muscle (skeletal and smooth), heart, pancreas, brain, lung, gonads, mesentery	Tight junctions (up to 2 nm) with continuous basement membrane, extravasation mainly by vesicular trafficking
Discontinuous endothelium Fenestrated Kidney glomeruli, GI tract mucosa, exocrine and endocrine glands, certain tumours, peritubular capillaries, choroid plexus, pancreas, intestinal wall	Interruptions (20 – 80 nm) between cell Junctions, membrane thickness 4 – 6 nm; continuous basement membrane
Sinusoidal Liver, spleen, red bone marrow, suprarenal and parathyroid glands, certain tumours, carotid and coccygeal bodies	Junctions up to 150 nm, basement membrane absent in liver and discontinuous in spleen and bone marrow

There are various important variations in the microvasculature bed, for example, arterioles, capillaries, and venules, that affect permeability. For example, venular portions of the capillaries have thin endothelial cell (170 nm), with frequent discontinuities of inter-endothelial. About 30% of venular junctions are believed to have gaps of about 6 nm. Arterioles, on the other hand, have endothelial cells that are liked by the tight junctions and communicating junctions, whereas the capillary endothelium contains only occluding junctions. Communicating gaps are small and rare in muscular venules and are absent in capillaries and pericytic venules. Endothelial cells in capillaries have more vesicles than those in arterioles (190 /μm^3 to 1000 /μm^3). The intercellular sealing is strong in arterioles, well developed in capillaries, and particularly loose in venules. Moreover, capillaries and venules have more trans-endothelial channels.

The macromolecules can travel across the endothelium by passive processes, such as nonspecific fluid-phase transcapillary pinocytosis and passage through inter-endothelial junctions, gaps, or fenestrae, or by receptor-mediated transport systems. Passive extravasation is affected by:

1) Regional differences in capillary structure,
2) The disease state of the tissue or organ,
3) The number and size of the microvascular surface area, and
4) Physicochemical properties of the macromolecules

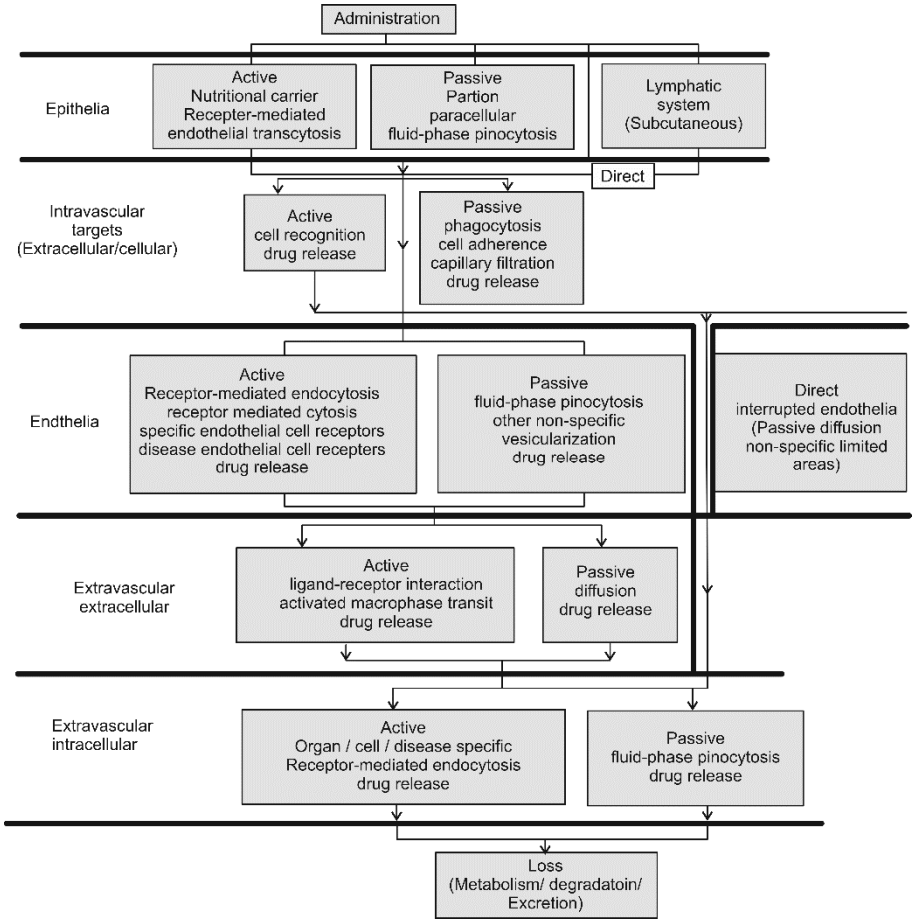

Fig.1.2 Anatomical and physiological pathways for site-specific drug delivery.

In general, the transfer of macromolecules across endothelium decreases progressively with an increase in molecular size. It is widely recognized that low molecular weight solutes and a large number of macromolecules, up to 30 nm in diameter, can cross the endothelium under some normal and pathophysiological conditions.

For proteins, the threshold restricting free passage through the glomerular endothelium is at a molecular weight of 60 000 to 70 000.

The molecules having molecular weight more than 70,000, are generally retained in the blood until these molecules are degraded and excreted. Some hydrophilic polymers, such as polyvinylpyrrolidone (PVP), dextran, polyethylene glycol (PEG), and N-(2-hydroxypropyl) meth-acrylamide (HPMA), exhibit much greater hydrodynamic radii, compared to proteins of same molecular weight restricting glomerular filtration is lower than for proteins (25,000 for PVP, 50,000 for dextran, 45,000 for HPMA)[39].

Since, the presence of anionic site on the endothelium and on the glycocalyx layer, anionic macromolecules show significantly slower rate of extravasation compared with neutral and cationic macromolecules. There may be the three-fold increase in the permeability of the pulmonary vascular system to cationic albumin, compared with negative albumin of the same molecular weight and hydrodynamic radius. Differences in the regions in the capillary structure and the number and size of the macrovascular surface area determine the flux of macromolecules in the interstitium. For example, organs such as the lung with very large surface areas will have a proportionately large total permeability and a high extravasation. Renal endothelium has a thick basement membrane, which contains anionic groups and heparin sulphate proteoglycan. Thus, extravasation through this membrane mainly depend on the molecular charge, shape, size, and lipophilic-hydrophilic balance characteristics of the macromolecules. Intestinal endothelium, although fenestrated, has been found to restrict mainly the passage of macromolecules. The absolute rate of extravasation varies considerably from one region to another within the alimentary canal. There is a large difference in permeation of solute macromolecules with a radius of less than 6 nm and no decrease in the permeation for molecules with a radius between 6 and 13.5 nm. The lung endothelium, which is non-fenestrated and vesicles with a size of 50 – 100 nm, is more selective to the passage of macromolecules; the lymph/plasma ratio was decreased from 0.7 to 0.25 nm when the molecular radius of the macromolecules increases from 3.7 to 11.0 nm[40]. Skeletal muscle, adipose tissue, liver, and myocardial endothelia show extravasation as a function of macromolecular size. The endothelium of the brain is the tightest of all endothelia in the body. It is formed by continuous, non-fenestrated endothelial cells and shows virtually no pinocytic activity. However, there are certain regions of the brain, for example, choroid plexus, that have fenestrated endothelium. Macromolecules such as horseradish peroxidase reach the cerebrospinal fluid by this route. Certain pathophysiological conditions, such as osmotic shocks, thermal injury, arterial hypertension, air or fat embolism, hypovolemia, and traumatic injury, cause transcapillary leakage and onset of pinocytic activity. This may have some implications in extravasation of macromolecules across the blood-brain-barrier.

The changes in the permeability of capillaries due to inflammation are believed to be due to the effect of histamine, bradykinin, and a variety of other mediators. In general, damaged capillaries show increased openings, ranging in size from 80 – 140 nm, in the endothelium and increase the transport activity. Macromolecules of up to 300,000 are capable of extravasation from blood vessels within experimental solid tumour, whereas molecules between 70,000 and 150,000 extravasate mainly from the vascular plexus induced around solid tumours. It is suggested that inflamed tissues show changes in the glycocalyx layer, which causes increased vesicular trafficking and,

increased extravasation of bloodborne materials. The metabolic changes are mediated through a reduced oxygen concentration, an increased concentration of carbon dioxide and a local increase in pH due to accumulation of various metabolites, affect extravasation.

Soluble macromolecules permeate the endothelial barrier more readily than particulate macromolecules. The rate of movement of fluid across the endothelium appears to be directly related to the difference between the hydrostatic and osmotic forces. Receptor-mediated transport systems include fluid-phase, constitutive, and non-constitutive endocytosis or transcytosis. It shows that particles having diameter smaller than 40 nm can enter these pathways. 5 nm gold-albumin particles are first adsorbed onto the specific binding sites of the endothelia in lung, heart, and diaphragm. These are then transported in transcytotic vesicles across the endothelium by receptor-mediated transcytosis and, to lesser extent, by fluid-phase processes. Low-density lipoproteins can pass through sinusoids.

- **Lymphatic Uptake**

After extravasation, drug molecules either be reabsorbed into bloodstream directly by the enlarged postcapillary inter-endothelial cell pores found in most tissues or enter into the lymphatic system and return with the lymph to the blood circulation. Lymph is a constituent of the interstitial fluid. The drugs administered by subcutaneous, intramuscular, transdermal, and peritoneal routes can reach the systemic circulation by the lymphatic system. A schematic representation of the integration of lymph and blood circulation. The lymphatic system originates in tissues as a network of fine capillaries. These capillaries coalesce regionally to form large vesicles called as afferent vessels, which extend centrally to one or more lymph nodes. The efferent ducts from the centrally located lymph nodes unite and form the major lymph trunks, for example, intestinal, cervical, and thoracic ducts, which finally coalesce with the venous supply at the root of the neck. Similar to blood capillaries, the lymphatic capillaries consist of a single layer of endothelial cells joined together by intercellular junctions. The diameter of small pores is 12 nm, whereas large pores range between 50 to 70 nm. The rate of formation of lymph depends on the hydrostatic pressure of blood and the permeability of the capillary wall. As blood enters the arterial end of the capillary, the hydrostatic pressure increases and, extravasation of water, electrolytes, and other bloodborne substances, for example proteins, occurs. By the time blood reaches the venular end of the capillary, the hydrostatic pressure drops, and some water and other low molecular weight, 10 000 substances are reabsorbed. However, there is a net excess of extravasation over reabsorption, which results in accumulation of excess lymph in the tissues. This accumulation of excess fluid causes an increase in the interstitial pressure, which forces the lymph to enter the lymphatic system. The larger lymphatic

vessels contain bicuspid valves, which prevent the retrograde flow of the lymph, while a coat of circular smooth muscle propels the lymph to flow centrally at a rate proportion to its rate of formation[41]. After absorption in the peripheral capillary bed, the lymph is transported by large lymph capillaries to the regional lymph node where lymphocytes are added. The lymph is then taken to the next node up the chain and, finally, into the great vein.

There are certain factors that control the clearance of drugs from interstitial sites, after extravasation or parenteral interstitial or transepithelial administration, include size and surface characteristics of particles, formulation medium, the composition and pH of the interstitial fluid, and disease within the interstitium. Studies indicate that soluble macromolecules smaller than 30 nm can enter the lymphatic system, whereas particulate matters larger than 50 nm are retained in the interstitial sites and serve as a sustained-release depot. The use of lipids or an oil in a formulation and the presence of a negative surface charge all appear to facilitate the absorption of particles into the lymphatic system. Generally, solid tumours do not have lymphatic drainage; the macromolecular drugs that enter tumour interstitium, by extravasation remain there. Commonly, this mechanism is called, the tumour enhanced permeability and retention effect (EPR). The drug trapped in the tumour interstitium may there be released, either intra- or extracellularly, by tumour associated proteolytic enzymes. The drug released then becomes capable of penetrating readily through cell membranes and reach its intracellular targets. In selective tumour therapy this concept can be used. The drug can be directly delivered into lymphatics as a potential approach to kill malignant lymphoid cells located in lymph nodes.

TUMOUR TARGETING

The incidence of malignant brain tumour (cancer) is 3.5 per 100,000 people across the world, and about 650 people are reported to be diagnosed with malignant brain tumours every day, this is as per the report of GLOBOCAN 2008[42]. Therefore, brain tumours have been threatening the human health severely due to fast development and poor prognosis. The most frequent primary brain cancer, called glioma, accounts for 29% of all primary brain and CNS tumours and 80% of malignant brain tumours[43]. A reason why poor prognosis and rapid recurrence are associated with the standard therapy is that the infiltrate growth of gliomas makes it difficult for the surgeon to completely remove pathologic or cancer-infiltrated tissues without affecting normal functions of the brain[44]. Moreover, failure is also attributed to the side effects of radiotherapy and poor outcome of usual chemotherapy. For a long time, researchers have tried hard to deliver therapeutic agents to the tumour region effectively and reduce unnecessary drug accumulation in normal brain and peripheral tissues. In fact, cancer is the second leading cause of death in U.S. and is the most challenging diseases to fight. For brain tumours, active

targeted drug delivery systems have attracted extensive attention in recent decades. In reality, the representative cytotoxic chemotherapeutic agents, such as paclitaxel, cisplatin, and doxorubicin, cannot distinguish cancer cells from normal cells. This lack of selectivity causes undesirable side effects associated with these drugs[45]. Since brain tumours have many distinctive characteristics from peripheral tumours due to their complicated oncogenesis, many factors are considered for effective brain tumour-targeted drug delivery, such as the barriers included in the whole process, the tumour microenvironment, and tumour cells. Currently, various targets have been exploited to achieve the targeted therapy using nanocarriers.

- **Blood-Brain-Barrier targeting strategies and related drug delivery system**

At the primary stage of development of brain tumour and at the infiltration growth regions of the tumour, the blood-brain barrier remains intact. BBB acts as a natural guard to protect the brain from harmful substances in the blood stream while supplying the brain with the necessary nutrients for proper function, is the important challenge for delivering drugs to brain tumour[46]. The blood-brain-barrier is a specialised system of capillary endothelial cells. These are partially covered by pericytes and basement membrane, and almost fully surrounded by the end feet of astrocytes. As a result, about 98% of the small molecules and 100% of large molecules including recombinant proteins and genes are prevented from being transported into the brain and reaching the tumour sites[47,48]. The blood-brain-barrier strictly restricts the transport of drug into the brain by serving as a physical (tight junctions), metabolic (enzymes) and immunological barrier.

This challenge can be tackled by many types of active targeting strategies which are used for developing effective drug delivery systems to the brain. This active targeting systems are mainly divided into (1) absorptive-mediated transcytosis (AMT), (2) transporter-mediated transcytosis, and receptor-mediated endocytosis (RMT)[49].

- **Absorptive-mediated transcytosis**

This provides a means for the delivery of drugs across the blood-brain-barrier by cationic proteins or cell-penetrating peptides (CPPs). It is triggered by electrostatic interactions between the positively charged moieties of the proteins and negatively charged membrane surface regions on the brain endothelial cells. Typical cationic bovine serum albumin-conjugated, pegylated nanoparticles (CBSA-NP) were prepared[50] for brain targeting. It was shown that the permeability of CBSA-NP was about 7.76 times higher than that of BSA-NP, which offered the possibility of delivering therapeutic agents to CNS. It reported that plasmid pORF-hTRAIL (pDNA)-incorporated CBSA-NP (CBSA-NP-hTRAIL) colocalized with glycoproteins in brain and tumour microvasculature and accumulated in tumour cells at 30 min after IV

administration to C6 glioma bearing nude mice, via absorptive-mediated transcytosis. Aclarubicin (ACL)-loaded cationic albumin conjugated pegylated nanoparticles (CBSA-NP-ACL) could significantly prolong the survival of the intracranial glioblastoma-bearing mice[51]. Other investigators[52] adopted another cationic protein, wheat germ agglutinin (WGA) conjugated to the surface of liposomes and also demonstrated enhanced BBB transport.

Moreover, alternative AMT-type cell-penetrating peptide (CPP)-based delivery systems show great ability in BBB transport. CPPs have been used to overcome the lipophilic barrier of cellular membrane and deliver a large variety of cargoes, including peptide/proteins. DNA/ oligonucleotide, antibodies, imaging agents, toxins, and nanodrug carriers such as liposomes and micelles. CPPs are heterogenous in size and sequence and are positively charged. Some share common features such as an amphipathic sequence and the ability to interact with lipid membranes. The CPPs are always derived from natural proteins including the transcription-activating factor Tat, penetration, and the Syn-B vectors, among which Tat might be the most frequently used[53].

- **Transporter-mediated transcytosis**

In the cerebral endothelium there are many kinds of transport systems, these provide the brain with the necessary nutrients and endogenous substances, transporter mediated transcytosis takes advantage of these transport systems as a promising brain targeting strategy. Transporter mediated transcytosis is substrate-selective, but only the drugs that are very similar to the endogenous carrier substrates will be taken up and transported into the brain. Receptor-mediated transcytosis is considered one of the most mature strategies for brain targeted drug delivery with the characteristics of high specificity, selectivity, and affinity. Although the ligand may have an effect on homeostasis and natural ligands may complete with the drug ligand to reduce targeting efficiency. Since many kinds of receptors are expressed on the capillary endothelium of the brain, such as transferrin receptor (TtR). The low density lipoprotein receptor (LDLR), the insulin receptor and nicotinic acetylcholine receptors, targeting ligands, including endogenous ligands and ligands based on phage-display or structure-guided design, have been used to facilitate receptor-mediated blood-brain-barrier transport of drug delivery systems.

One of the most widely characterized receptor-mediated transcytosis systems for brain targeting is the transferrin receptor (TtR). The transferrin receptor is highly expressed on endothelial cells of blood-brain-barrier[54]. A transferrin-conjugated drug delivery system for blood-brain-barrier delivery has been developed. The transferrin-modified paclitaxel-loaded polyphospho-ester hybrid micelles (TPM) was prepared to measure the *in vitro* and *in vivo* brain-targeting efficiencies. It has been found that TPM showed stronger anti-glioma activity and the mean survival time of mice bearing intracranial U87

MG glioma was significantly prolonged. However, Tf is not likely an ideal brain delivery ligand since the Tf-modified targeted drug delivery system would have to complete with the natural ligang[55]. Similarly, mouse monoclonal antibody (MAb) against the rat TtR, OX26, has been extensively examined. When OX26 is coupled with the liposomes, transferrin receptor-mediated targeting of daunomycin to the rat brain was achieved by using an immunoliposome-based drug delivery system[56]. Another common receptor, the low-density lipoprotein (LDL) receptor-related protein (LRP), has been said to mediate to mediate transport of various ligands conjugated to nanocarriers across the blood-brain-barrier. Aprotinin is a LRP ligand. Its ability to transport blood-brain-barrier has been evaluated using an *in vitro* model of the blood-brain-barrier and *in situ* brain perfusion. Its transcytosis across bovine brain capillary endothelial cell monolayers was at least 10-fold more than that of holo-transferrin. Angiopep, derived from aprotinin with the Kunitz domains of human proteins, exhibited higher transcytosis capacity and accumulation of parenchymal. Angiopep-2 modified cationic liposomes for the efficient co-delivery of a therapeutic gene with paclitaxel to the brain. After treatment with liposomes, the median survival time of brain tumour-bearing mice was found significantly longer than that of other groups. Thus, it was thought a promising drug delivery strategy against glioma.

Nicotinic acetylcholine receptors (nAChRs) are a type of ligand-gated ion channel that is widely expressed in the brain including the brain capillary endothelial cells[57]. Since, they bind the second loop of the three-finger snake toxin with high affinity and selectivity. nAChRs could be used to facilitate blood-brain-barrier crossing and intracranial transport of drug delivery systems. It has been reported that a 29-amino acid peptide derived from rabies virus glycoprotein-RVG29 enabled transvascular siRNA delivery to the brain through nAChRs and provided a safe and non-invasive method for delivery of therapeutic agents across the blood-brain-barrier. Thus, nAChR-mediated brain targeting would be a promising strategy for the intracranial transport of drug delivery systems. A 16 amino acid peptide CDX, derived from the loop II region of the snake neurotoxin candoxin exhibited high binding affinity to nicotinic acetylcholine receptors. This peptide enabled significantly prolonged the survival time of intracranial glioblastoma-bearing mice[58].

BRAIN SPECIFIC DRUG DELIVERY

In fact, in the central nervous system, a direct administration of drugs to the CNS can achieve targeted action[59]. Blood-brain-barrier can effectively damage the effect of the large number of drugs for example, antibiotics, antineoplastic agents and neuropeptides-CNS stimulant drug because of its stubborn hindrance effect[60]. Some recent studies indicate that the blood-brain-barrier commonly does not cross about 100% of large molecule drugs and about 98% of small molecule drugs[61]. Currently, various methods have been

developed with increased pharmacodynamic effects for the treatment of brain disorders[62]. Drug discovery and drug delivery technology are the two main areas where advancement is necessary to deliver drug to the brain. Nanoparticles drug delivery system (NDDS) is one of the such advanced and effective technology against various CNS disorders.

Drug delivery means the methods, formulation technologies and systems for transporting a pharmaceutical compound in the body so that the therapeutic effect is safely achieved. It may involve scientific site-targeting within the body or it might involve facilitating systemic pharmacokinetics. It is typically concerned with both quantity and duration of presence of drug. Drug delivery methods modify drug release profile, absorption, distribution and elimination for the benefit of improving efficacy of product and safety, and patient convenience. Efforts in the area of drug delivery include the development of targeted delivery in which the drug is active in the target area of the body. The goal of a targeted drug delivery system is to prolong, localize, target and have a protected drug interaction with the diseased tissue. Targeted drug delivery system have been developed to optimize regenerative techniques. This maintains the required plasma and tissue drug levels in the body, thus, preventing any damage to the healthy tissue through the drug. Barriers in brain Targeted Drug Delivery refers to the failure of systemically delivered drugs to effectively treat many CNS diseases can be rationalized by considering a number of barriers that inhibit drug delivery to the CNS. There are physical barriers that can separate the brain extracellular fluid from the blood.

Factors influencing drug delivery to brain:

- Blood brain barrier
- Cerebrospinal fluid
- Physicochemical factors

To bypass the blood-brain-barrier; to deliver the therapeutic effects to the brain. The time for developing CNS drugs is normally much longer than for non-CNS drugs. Clinical trials of CNS drugs become challenging because of the complexity of the brain, side effects and the impermeable blood-brain-barrier[63].

Approaches

Invasive, Pharmacological and Physiological.

- Invasive: Intracerebro ventricular, convention enhanced polymer/ microchip implants.
- Pharmacological: Some of the wet molecules can freely enter such as alcohol, nicotine, benzodiazepine of molecular size C 500 D.
- Physiological: Used to increase the brain delivery, transcytosis capacity for example, receptor mediated transcytosis, the drugs are lipophilic. A drug

must exhibit high potency and selectivity towards the biological target, and reach a concentration above an experimentally determined threshold within brain tissue.

Functions of blood-brain-barrier (BBB)

There are various functions of blood-brain-barrier[65].

- It works as a pump
- It stands in the form of a metabolic barrier

Regulatory function of BBB are

- Protecting the brain from foreign substances in blood that may injure the brain
- Protection of the brain from hormones
- Neurotransferance in the rest of the body

Presently, worldwide brain diseases affect one in six people, and they include a wide range of neurological diseases from Alzheimer's and Parkinson's diseases to epilepsy, brain injuries, brain cancer, neuro-infections and strokes. The treatment of these diseases is complex and limited due to the presence of the blood-brain barrier, which covers the brain entirely. The blood-brain barrier protects not only the brain from the harmful substances but also provides a metabolic barrier and regulates the transport of nutrients/serum factors/ neurotoxins. When the brain diseases are treated, it makes it easy to understand the lack of efficacy of therapeutic drugs, resulting from the innate resistance of the BBB to permeation. To overcome this limitation, drug delivery systems based on nanotechnology /microtechnology have been wisely developed. Brain-targeted drug delivery allows targeted therapy with a higher therapeutic efficacy and low side effects because it targets moieties present in the drug delivery systems. Brain-targeted drug delivery research is an active, rich and multidisciplinary research area. The current survey reveals the topics of

i) Novel drug delivery systems for GBM treatment,
ii) The potential of Alzheimer's disease immunotherapy, and lastly,
iii) Current methods to detect and monitor macromolecules in the brain.

The main problem to treating disorders of the central nervous system (CNS) is the presence of the BBB, which hinders the delivery of therapeutics. It is known that few small molecule drugs can cross the blood-brain barrier, and most of the biologic drugs cannot. As an alternative route to overcome the blood-brain barrier. The biodistribution of an anti-EGFR antibody was evaluated in the rat brain after intra-cisterna magma injection. They show vastly greater and deeper penetration of the monoclonal antibody (mAb) into the brain parenchyma after CSF administration compared to IV administration. It demonstrates that circumventing the blood-brain barrier via

CSF microcirculation might be a strategy to improve the delivery of mAbs into the brain, achieving deep penetration of IgG-size biologics. Another route of administration that allows to successfully reach the brain is the intranasal route. Intranasal administration of drugs can reach the brain, this route bypasses the blood-brain barrier through olfactory bulb. This route is used to enhance gene delivery to the cerebral cortex using hyaluronidase-coated glycol chitosan-DNA polyplexes (GCPH). It has been found that high levels of protein expression in the brain regions upon intranasal administration of hyaluronidase-coated polyplexes. Following the same strategy of intranasal administration developed a naringenin-encapsulated nanostructured lipid carrier (NGN-NLC) with thymoquinone (TQ) oil to investigate the antidepressant potential of the nanosystem. Their *ex vivo* and *in vivo* results show higher penetration and higher penetration and greater antidepressant potential from NGN-NLC compared to NGN suspension achieved by intranasal administration. Lastly, a pomegranate seed oil (PSO) phospholipid oil gel for nasal administration to test its biological effect on memory and locomotor activity. The results show a significant improvement in the behaviour of animals when they were treated with intranasal gel compared to orally administered oil. Another common route of administration for brain-targeted drug delivery is intracranial. The novel peptide-toxin conjugate that binds to IL-13Rα2 was able to significantly decrease size of tumour and prolong survival in diffuse midline and high-grade glioma with high levels of IL-13Rα2, opening doors for IL-13Rα2-targeted therapy. Intravenous administration for brain-targeted drug delivery has been focused and hollow-gold nanoparticles were developed bounded to liposomes (HGN-liposomes) loaded with muscimol to be released by laser or ultrasound stimulation and to inhibit neurons and supress epileptiform seizures[66]. Combination of ultrasound stimulation and intravenous administration of HGN-liposomes supressed seizure activity in the hippocampus. This demonstrates the therapeutic potential of HGN-liposomes for controlling epileptiform seizures without continuous exposure. By intraperitoneal administration, the drug is delivered into the brain can also be done. PLGA microparticles and nanoparticles with Tolcapone have been developed to improve the treatment of Parkinson's disease (PD)[67]. An urgent need was felt to find new and promising therapeutic strategies to treat PD that can overcome the blood-brain barrier. It has been demonstrated that Tolcapone-loaded PLGA nanoparticles can revert PD-like symptoms of neurodegeneration in an *in vivo* model upon intraperitoneal administration. Undoubtedly, the blood-brain barrier is essential for protecting the brain from toxins, drugs, and pathogens, and this serves as a highly selective semipermeable membrane of endothelial cells. Damaging the blood-brain barrier can lead to serious consequences for brain homeostasis and neuronal degeneration. The impact of the impact of high-dose acetaminophen (APAP) on the integrity of the blood-brain barrier, this

demonstrates increased paracellular permeability of the blood-brain barrier and increased protein expression of claudin-5 in brain micro vessels. It has been observed that APAP-induced paracellular 'leak' contributed to increased CNS uptake of codeine. This brings awareness to the biological effects of concomitant administration of APAP with opioids.

References

1. Ehrlich, P.; John Wiley & Sons, London, 1906
2. Tomlinson, E.; J. Pharm. Pharmacol.; 44 (Suppl.1), 147, 1992
3. Tomlinson, E.; Int. J. Pharm. Technol. Prod. Manuf.; 4, 49,1983
4. Wider, K.J., Senyei, A.E., and Ranney, D.F.; Adv. Pharmacol. Chemother.; 16, 213, 1979.
5. Tomlinson, E.; (Patho)physiology and the temporal spatial aspects of drug delivery, in *Site-Specific Drug Delivery. Cell Biology, Medical, and Pharmaceutical Aspects* (Tomlinson, E., Davis, S.S., eds); John Wiley & Sons.; Chichester; 1986.
6. Vanoss, C.J., Gilman, C.F., Bronson, P.M. and Border, J.R.; Immunol. Commun.; 3, 329, 1974.
7. Davis, S.S., Douglas, S.J., Illum, L., Jones, P.D.E., Mak, E., and Muller, R.H.; Targeting of colloidal carriers and the role of surface properties, in *Targeting of Drugs with Synthetic Systems* (Gregoriadis, G., Senior, J., and Poste, G., eds.). Plenum Press, New York, 1986.
8. Illum, L., Davis, S.S., Jones, P.D.E.; Polym. Preprints; 27, 25, 1986.
9. Illum, L. and Davis, S.S.; Life Science; 40, 1553, 1987.
10. Saito, K., Ando, J., Yoshida, M., Haga, M., and Kato, Y.; Chem. Pharm. Bull.; 36, 4187, 1988.
11. Moghimi, S.M., Porter, C.J., Muir, I.S., Illum, L., and Davis, S.S.; Biochem. Biophys. Res. Commun.; 177, 861, 1991.
12. Hsu, M.J. and Juliano, R.L.; Biochim. Biophys. Acta.; 720, 411, 1982.
13. Hopkins, C.R.; Site-specific drug delivery – cellular opportunities and challenges, in *Site-Specific Drug Delivery, Cell Biology, Medical and Pharmaceutical Aspects* (Tomlison, E., and Davis, S.S., eds); John Wiley & Sons.; Chichester; 1986.
14. Duncan, R., Cable, H.C., Rypacek, F., Drobnik, J., and Lloyd, J.B.; Biochem. Soc. Trans.; 12, 1064, 1984.
15. Duncan, R., Cable, H.C., Rejmanove, P., and Kopecek, J.; Biochim. Biophys. Acta.; 799, 1, 1984
16. Kooistra, T., Duursma, A., Boma, J.M.W., and Gruber, M.; Biochim. Biophys. Acta.; 631, 439, 1980

17. Curran, P.F., Mcintosh, J.R.; A model system for biological water transport. Nature (Lond) 193:347—348, 1973
18. Curran, P.F., Schultz, S.G.; Transport across membranes: general principles, in Handbook of Physiology, Section 6, The Alimentary Canal: vol. III Intestinal Absorption, edited by CODE CF, Washington, D.C., Am Physiol Soc, 1968, pp. 12 17—1243
19. Patiak, C.S., Goldstein, D.A., Hoffman, I.F.; The flow of solute and solvent across a two membrane system.; J Theor Biol; 5, 426—442, 1963
20. Diamond, J.M., Bossert, W.H.; Standing-gradient osmotic flow; A mechanism for coupling of water and solute transport in epithelia; Gen Physiol; 50, 2061—2083, 1967
21. Sackin, H, Boulpaep, E.L.; Models for coupling of salt and water transport: Proximal tubular reabsorption in Necturus kidney. J Gen Physiol, (1975) 66 (6), 671–733
22. Harris, A.S.; Biopharmaceutical aspects of the intranasal administration of peptides, in Delivery Systemsfor Peptides (Davis, S.S., Illum, L., and Tomlinson, E., eds); Plenum Press, New York; 1986.
23. Illum, L., Farraj, N.F., Critichley, H., and Davis, S.S.; Int. J. Pharm.; 46, 261, 1988.
24. Harmans, W.A.J.J., Deurloo, M.J.M., Romeyn, S.G., Verhoef, J.C., and Merkus, F.W.H.M.; Pharm. Res.; 7, 500, 1990.
25. Fisher, A.N., Farraj, N.F., O'Hagan, D.T., Jabbal-Gill, I., Johansen, B.R., Davis, S.S., and Illum, L.; Int. J. Pharm.; 74, 147, 1991.
26. Pusztai, A.; Adv. Drug Deliv. Rev.; 3, 215, 1989
27. Matsuno, K., Schaffner, T., Gerbel, H.A., Ruchti, C., Hess, M.W., and Cottier, H.; Reticuloendothel. Soc.; 33, 263, 1983.
28. Damge, G., Aprahamian, M., Balboin, G., Hoetzel, A., Andrieu, V., and Devissaguet, J.P.; Int. J. Pharm.; 36, 121, 1987.
29. Tomlinson, B.; Adv. Drug Devil. Rev.; 1, 87, 1987
30. Ritschel, W.A.; Methods Find. Exp. Clin. Pharmacol.; 13, 205, 1991
31. Dressman, J.B., Bas, P., Ritschel, W.A., Friend, D.R., Rubinstein, A., and Zhiv, B.; J. Pharm. Sci.; 82, 857, 1993.
32. Ritschel, W.A.; Methods Find. Exp. Clin. Pharmacol.; 13, 313, 1991
33. Lee, V.H.L., and Yamamoto, A.; Adv. Drug Deliv. Res.; 4, 171, 1990
34. Ahsan CR, Hajnoczky G, Maksymowych AB, Simpson LL. 2005. Visualization of binding and transcytosis of botulinum toxin by human intestinal epithelial cells. J Pharmacol Exp Therapeut 315: 1028–1035.
35. Albac S, Schmitz A, Lopez-Alayon C, d'Enfert C, Sautour M, Ducreux A, Labruere-Chazal C, Laue M, Holland G, Bonnin A, et al. 2016.

Candida albicans is able to use M cells as a portal of entry across the intestinal barrier in vitro. Cell Microbiol 18: 195–210.

36. Ang SF, Folsch H. 2012. The role of secretory and endocytic pathways in the maintenance of cell polarity. Essays Biochem 53: 29–39.
37. Apodaca G, Katz LA, Mostov KE. 1994. Receptor-mediated transcytosis of IgA in MDCK cells is via apical recycling endosomes. J Cell Biol 125: 67–86.
38. Apodaca G, Gallo LI, Bryant DM. 2012. Role of membrane traffic in the generation of epithelial cell asymmetry. Nat Cell Biol 14: 1235–1243.
39. Azizi PM, Zyla RE, Guan S, Wang C, Liu J, Bolz SS, Heit B, Klip A, Lee WL. 2015. Clathrin-dependent entry and vesicle-mediated exocytosis define insulin transcytosis across microvascular endothelial cells. Mol Biol Cell 26: 740 –750.
40. Baker K, Qiao SW, Kuo T, Kobayashi K, Yoshida M, Lencer WI, Blumberg RS. 2009. Immune and non-immune functions of the (not so) neonatal Fc receptor, FcRn. Semin Immunopathol 31: 223–236.
41. Barroso M, Sztul ES. 1994. Basolateral to apical transcytosis in polarized cells is indirect and involves BFA and trimeric G protein sensitive passage through the apical endosome. J Cell Biol 124: 83–100.
42. Ferlay, I., Shin, H.R., Bray, Forman, D., Mathers, C., Parkin, D.M.; Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008; Int. J. Cancer; 127, 2893 – 2917, 2010.
43. Dolecek, T.A., Propp, J.M., Stroup, N.E., Kruchko, C.; CBTRUS statistical report: primary brain and central nervous system tumours diagnosed in the United States in 2005 – 2009; Neuro Oncol; 14, 49, 2012.
44. Sanai, N., Berger, M.S.; Glioma extent of resection and its impact on patient outcome; Neurosurgery; 62, 753 – 764, 2008.
45. Ojima, I., Geng, X., Wu, X.,Qu, C., Borella, C.P., Xie, H., Wilhelm, S.D., Leece, B.A., Bartle, L.M., Goldmacher, V.S., and Chari, R.V.J.; Tumour-specific novel taxoid-monoclonal antibody conjugates; J. Med. Chem.; 45, 5620 – 5623, 2002.
46. Daneman, R.; The blood-brain barrier in health and disease; Ann Neurol; 72, 648 – 672, 2012.
47. de Boer, A.G., Gaillard, P.I.; Drug targeting to the brain; Ann Rev Pharmacol Toxicol; 47, 323 – 355, 2007.
48. Wong, A.D., Ye, M., Levy, A.F., Rothstein, J.D., Bergles, D.E., Searson, P.C.; The blood-brain barrier: an engineering perspective.; Front Neurveng; 6, 7, 2013.

49. Pardridge, W.M.; Drug targeting to the brain; Pharm Res; 24, 1733 – 1744; 2007
50. Lu, W., Tan, Y.Z., Hu, K.I., Jiang, X.G.; Cationic albumin conjugated pegylated nanoparticle with its transcytosis ability and little toxicity against blood-brain barrier; Int. J. Pharm.; 295, 247 – 260, 2005.
51. Misra, A., Ganesh, S., Shahiwala, A., and Shah, S.P.; "Drug delivery to the central nervous system: a review", J Pharm Pharm Sci, Vol. 6, pp. 252-73,2003.
52. Schinkel, A. H., Wagenaar, E., Mol, C., and van Deemter, L. ; "Pglycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs", Journal of Clinical Investigation, Vol. 97, pp. 2517,1996.
53. Pardridge, W. M.; "Blood-brain barrier drug targeting: the future of brain drug development", Molecular interventions, Vol. 3, pp. 90,2003.
54. Löscher, W., and Potschka, H.; "Blood-brain barrier active efflux transporters: ATP-binding cassette gene family", NeuroRx, Vol. 2, pp. 86-98,2005
55. Lingineni K, Belekar V, Tangadpalliwar SR, et al. The role of multidrug resistance protein (MRP-1) as an active efflux transporter on blood-brain barrier (BBB) permeability, Mol Divers. 2017; 21:355–65.
56. Davis, S.S.; J. Pharm. Pharmacol.; 44(Suppl. I), 186, 1992
57. Merkle, H.P., Anders, R., Sandow, J., and Schurr, W.; Drug delivery of peptides: The buccal route, in *Delivery Systems for Peptide Drugs* (Davis, S.S., Illum, L., and Tomlinson, E., eds); Plenum Press, New York, 1986.
58. Ritschel, W.A., Ritschel, G.B., Ritschel, B.E.C., and Lucker, P.W.; Methods Find. Exp. Clin. Pharmacol.; 10, 645, 1988
59. Richardson, J.L., Minhans, P.S., Thomas, N.W., and Illum, L.; Int. J. Pharm.; 16, 29, 1989
60. Taylor, A.E., and Granger, D.N.; Exchange of macromolecules across the microcirculation, in *Handbook of Physiology*, 6, *Microccirculation*, Part I (Renkin, E.M., and Michel, C.C., eds); Waverly Press, Baltiimore, 1984.
61. Hall, J.G.; The lymphatic system in drug targeting: An overview, in *Targeting of Drugs*. *Anatomical and Physiological Considerations* (Gregoriadis, G., and Poste, G., eds); Plenum Press, New York, 1985.
62. Lingineni K, Belekar V, Tangadpalliwar S.R., et al. The role of multidrug resistance protein (MRP-1) as an active efflux transporter on blood-brain barrier (BBB) permeability, Mol Divers. 2017; 21:355–65.

63. Sun, Y.; The focal spread of macromolecular tracers in vessel walls: Frequency and effect of intima compaction and blood pressure", Sun, Y. Vol. 2008: ProQuest.
64. Dixit, K., Athawale, R.B. and Singh, S.; "Quality control of residual solvent content in polymeric microparticlesJournal of microencapsulation, pp. 1-16,2014.
65. Naseri Kouzehgarani, G.; Kumar, P.; Bolin, S.E.; Reilly, E.B.; Lefebvre, D.R. Biodistribution Analysis of an Anti-EGFR Antibody in the Rat Brain: Validation of CSF Microcirculation as a Viable Pathway to Circumvent the Blood-Brain Barrier for Drug Delivery. Pharmaceutics 2022, 14, 1441.
66. Groothuis, D.R.; "The blood-brain and blood-tumor barriers: a review of strategies for increasing drug delivery", Neuro-oncology, Vol. 2, pp. 45-59,2000.
67. Mash, D.C. and Moriarty, R.M.; "Compositions comprising noribogaine and an excipient to facilitate transport across the blood brain barrier", 2014, Google Patents.

Exercises

Multiple Choice Questions

1. Recombinant DNA is
   a. Naturally available DNA
   b. Formed by RNA molecules
   c. Called chimeric DNA
   d. The DNA ready for combination
2. Nonspecific pinocytic uptake appears to be depending on
   a. Configuration,
   b. Charge,
   c. Hydrophobicity of the pinocytic substrates
   d. All of the above
3. The different order of sensitivity of different regions of GI tract can be shown as:
   a. Rectum > colon > small intestine > stomach.
   b. Colon> small intestine > stomach > rectum
   c. Stomach > rectum > colon > small intestine
   d. Small intestine > stomach > rectum > colon

4. Which of following are internally lined with one or more layers of epithelial cells?
   a. buccal,
   b. vaginal, and
   c. rectum
   d. All the above
5. In a delivery system containing a carrier must be…….
   a. Biodegradable
   b. Readily eliminated without any problem
   c. All the above
   d. None of the above
6. Average length of a human rectum is……
   a. 10 – 20 cm
   b. 15 – 19 cm
   c. 1.5 – 2.0 cm
   d. 1.0 – 2.0 cm
7. Based on which the blood capillaries are divided into three types, such as continuous, fenestrated, and sinusoidal?
   a. Morphology and continuity of the endothelial layer and the basement membrane
   b. Distribution and presence
   c. Internal structure of the location
   d. None of the above
8. Which of the following statement is correct?
   a. Continuous capillaries are not common and widely distributed in the body.
   b. Continuous capillaries are common and widely distributed in the body.
   c. Continuous capillaries are common but not widely distributed in the body.
   d. Continuous capillaries are used commonly to constitute the tissues.

9. Which of the following statements is correct?
   a. Fenestrated capillaries show inter-epithelium gaps of 20 – 80 nm at irregular intervals.
   b. Fenestrated capillaries show inter-endothelium gaps of 20 – 80 nm at regular intervals.

c. Fenestrated capillaries show inter-endothelium gaps of 20 – 80 nm at irregular intervals.
d. Fenestrated capillaries show inter-endothelium gaps of 20 – 80 nm at irregular intervals and these gaps have thick membrane.

10. Which of the following statements is correct?
    a. Endothelial cells in capillaries have limited vesicles in the arterioles
    b. Endothelial cells in capillaries do not have any vesicles in the arterioles
    c. Endothelial cells in capillaries have less vesicles than those in arterioles
    d. Endothelial cells in capillaries have more vesicles than those in arterioles
11. Which of the following statements is correct ?
    a. Sinusoidal capillaries are wider in diameter,
    b. Sinusoidal capillaries have an regular lumen,
    c. The wall of sinusoidal capillaries is very thick.
    d. Sinusoidal capillaries are narrower in diameter
12. Passive extravasation is affected by
    a. The number and size of the microvascular surface area,
    b. The number and size of the microvascular surface area
    c. Physicochemical properties of the macromolecules
    d. All the above
13. The macromolecules are widely recognized for ……
    a. Low molecular weight solutes
    b. A large number of macromolecules, up to 30 nm in diameter
    c. All the above
    d. None of the above
14. Which of the following is correct?
    a. The macromolecules can cross the epithelium tissues under normal conditions.
    b. The macromolecules can cross the endothelium under some normal and pathophysiological conditions.
    c. The macromolecules can cross the endothelium only under pathophysiological conditions.
    d. The macromolecules cannot cross the endothelium under some normal and pathophysiological conditions.

15. Which of the following can cause transcapillary leakage and onset of pinocytic activity?
    a. Only transcapillary leakage
    b. Transcapillary leakage and onset of pinocytic activity
    c. No onset of pinocytic activity
    d. No transcapillary leakage and pinocytic activity
16. What are certain factors that control the clearance of drugs from interstitial sites, after extravasation or parenteral interstitial or transepithelial administration?
    a. Size and surface characteristics of particles,
    b. The composition and pH of the interstitial fluid.
    c. All the above
    d. None of the above
17. The brain tumours can threaten the human health severely due to
    a. Slow development
    b. Excessive prognosis.
    c. Extensive attention
    d. Fast development and poor prognosis
18. The statement given below is true (T) or false (F) ?
    a. The brain tumours have many distinctive characteristics from peripheral tumours
    b. T
    c. F
    d. None
19. Regulatory function of blood brain barrier are ……
    a. Neuro transferance in the rest of the body
    b. Protection of the brain from hormones
    c. All the above
    d. None of the above
20. The novel peptide-toxin conjugate that binds to IL-13Rα2 was able to significantly decrease size of tumour and prolong survival in diffuse midline and high-grade glioma with ……
    a. Medium levels of IL-13Rα2
    b. High levels of IL-13Rα2
    c. Low levels of IL-13Rα2
    d. Very low levels of IL-13Rα2

Short Questions

1. What is target specific drug delivery?
2. What are the requirements for drug targeting?
3. What are the different classes of target oriented drug delivery with suitable examples?
4. What are the methods used for targeted drug delivery?
5. Passive extravasation can be influenced by what?
6. What is brain-specific drug delivery?
7. What is blood-brain-barrier?
8. What are the functions of blood brin barrier?
9. What are different methods involved in drug targeting?
10. What are different classes of drug targeting?

Long Questions

1. Explain why 'target specific drug delivery' is better than 'conventional oral delivery'.
2. What are different methods of drug targeting? Explain briefly the effects of biological processes in drug targeting.
3. Explain in brief the Absorptive-mediated transcytosis.
4. What is meant by Transporter-mediated transcytosis? Write down the process of transporter-mediated transcytosis.
5. What do you understand by cellular uptake? Explain in brief the cellular uptake and processing.
6. Explain in brief the Absorptive-mediated transcytosis.
7. What are the approaches involved in drug targeting to the brain? Describe in short targeting to tumours.
8. Write a note on brain specific drug delivery.
9. What is meant by BBB? Explain in short under what conditions a drug be targeted to the brain?
10. Explain the functions of blood-brain-barrier.

Answers

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
c	d	a	d	c	b	a	b	c	d	a	d	c	b	b	c	d	a	c	b