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Review Article

Targeting strategies and nanocarriers in vaccines and therapeutics

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In the past few decades, remarkable advances have been made in the field of immunology and molecular biology. Even though the efficacy level, protein binding capacity and other pharmacological parameters are extraordinary, formulations have become more challenging in terms of making drugs or antigens reach specific sites of action, the release rate of a drug at the site of action, proper presentation of an antigen by antigen-presenting cells or dendritic cells and other pharmacokinetic and pharmacodynamic parameters of finished drug products and vaccines. The purpose of this review is to present a brief overview of the challenges to drug targeting, especially vaccines, as well as of different approaches designed to overcome these barriers.

Key words: Dendritic cells, drug delivery, nanoparticles, pharmacology, therapy, vaccines

Abbreviations: APC- antigen-presenting cell; AET- active efflux transport; BBB- blood-brain barrier; BCSFB- blood cerebrospinal fluid barrier; bFGF- basic fibroblast growth factor; CDS- chemical drug delivery system; CNS- central nervous system; CMT- carrier-mediated transport; DC- dendritic cell; LDL- low density lipoprotein; MBB- marrow blood barrier; MAO- monoamine oxidase; MRP- multi-drug resistance; MW- molecular weight; NLS- nuclear localization sequence; PEG- polyethylene glycol; RES- reticuloendothelial system; RMT- receptor mediated transport; TDDS- targeted drug delivery system; VEGF- vascular endothelial growth factor

The concept of a targeted drug delivery system (TDDS), which was first proposed by Paul Ehrlich in 1902, in which he called the hypothetical drug a magic bullet, is still under extensive research (Ehrlich, 1902; Strebhardt and Ullrich, 2008; Patel *et al*. 2010). This targeted drug delivery system (TDDS) releases its drug at a preselected biosite in a controlled manner. Recent knowledge to explore drug delivery systems has reached a certain level where scientists are clear about the pathophysiology of most diseases, as well as the physiological and anatomical interventions of the body's systems (Xiao *et al*. 2002; Reichel, 2006; Bae and Park, 2011; Madhuri *et al*. 2011). Furthermore, drug-targeting challenges have become more sophisticated in terms of more efficacy, less

toxicity of the drug design, higher immunogenicity of vaccines and higher expression levels of gene therapy (Morenweiser, 2005; Patil *et al*. 2005; Madhuri *et al*. 2011; Sarkar and Suresh, 2011; Malabadi *et al*. 2011). The introduction of the carrier system concept in recent years has opened up a new era for drug targeting (Bae and Park, 2011). For example, activation of dendritic cells (DCs) through a nanocarrier-based system has made it possible to efficiently create and develop immunogenic DNA vaccines (Xiang *et al*. 2010). DCs play a critical role in the generation of the antigen-specific antiviral and antitumor T cell immune responses (Inaba *et al*. 1995; Jiang *et al.* 1995; Banchereau and Steinman, 1998; Den Haan *et al*. 2000; Nestle *et al*. 2001; Cerundolo *et al*. 2004; van Broekhoven *et al*. 2004; Mahnke *et al*. 2005; Tacken *et al.* 2006; Wang *et al*. 2005, 2009). DCs present in peripheral tissues are immature but sufficiently able to respond to dangerous signals by expressing various surface receptors (maturation), indicating the presence of an infection (Inaba *et al*. 1995; Jiang *et al.* 1995; Banchereau and Steinman, 1998; Den Haan *et al*. 2000; Nestle *et al*. 2001; Guermonprez *et al*. 2002; Cerundolo *et al*. 2004; Wang *et al*. 2005, 2009). Scientists are currently investigating different types of nanocarrier systems such as multiple emulsions, liposomes, and polymeric nanoparticles as delivery systems to achieve different levels of formulation goals (Shahiwala *et al.* 2007).

TARGETING CHALLENGES FOR VACCINES AND THERAPEUTICS

Successful drug targeting involves a clear understanding of various distributional and rate processes, as well as drug metabolism and disposition (Rapaka, 1995; Mizuno *et al*. 2003). Drug distribution and rate processes depend on the nature of biological and cellular membranes, the distribution of drug receptors, local blood flow, enzyme system, etc. (Rapaka, 1995; Mizuno *et al*. 2003). Scientists have already achieved a benchmark in physiology and molecular biology which demonstrates a number of aspects that are at a standstill remain challenging for drug targeting (Rapaka, 1995; Mizuno *et al*. 2003). Among them, brain targeting for vaccines and therapeutics is one of the major challenging areas for scientists due to its outstanding anatomical and physiological features (Mizuno *et al*. 2003; de Boer and Gaillard, 2007). These are: (1) Bone skull, an anatomical barrier for transdermal delivery systems; (2) Blood-brain barrier (BBB), a physiological barrier for less lipophilic and large molecular sized proteins as only small and lipid-soluble drugs can penetrate across the BBB; (3) Blood cerebrospinal fluid barrier (BCSFB), a biochemical barrier for vaccines and therapeutics (Mizuno *et al*. 2003; de Boer and Gaillard, 2007; Pavan *et al*. 2008).

For drugs (or vaccines) whose targets are located in the cytoplasm or nucleus of a cell, the challenge to physiological barriers is the need to diffuse through the viscous cytosol to access particular cytoplasmic targets where the site of action is located (Mozafari, 2006). A barrier such as the nuclear membrane is a formidable challenge for the passage of oligonucleotides, plasmid DNA, etc. (Mozafari, 2006). The viscous cytosolic biochemical barrier is another challenge for drug candidates like peptides since chemical drug molecules have peculiar biochemical composition of different types of enzymes (Mozafari, 2006).

The rapid clearance of foreign particles from circulation by macrophages lining the sinusoids in the liver, spleen and bone marrow is one of the most important mechanisms in host defense against infections (Staub, 1994). The distinct physiological functions of the components of the bone marrow make it challenging for drug targeting, gene therapy or vaccine targeting

(Staub, 1994). This distinct feature is due to sinuses, a relatively large blood vessel forming a barrier called the marrow blood barrier (MBB) (Staub, 1994). Its selective uptake of molecules from the circulation system into the reticuloendothelial system (RES) of bone marrow offers a tremendous challenge for delivery system formulations (Vyas and Khar, 2002). The majority of pathogens invade the body cavity through one of the mucosal routes that cover the aero-digestive or urinogenital tract, eye conjunctiva, inner ear and the ducts of all endocrine glands (Staub, 1994; Vyas and Khar, 2002). These areas where almost 80% of all immunocytes are present are treated as the first line defense system of a body. In traditional vaccination, where whole cells or attenuated whole cells are generally used as vaccine formulation with adjuvants, mucosal routes are prime targets (Ongkudon *et al*. 2011). For these mucosal vaccines a number of challenges have to be faced by formulation scientists. Furthermore, adequate binding of antigen to the target cells for proper presentation depends on proper attachment and colonization, which is challenging for non-invasive bacteria as well as penetration and replication, which are further challenging for invasive bacteria and viruses (Ongkudon *et al*. 2011). A new concept that has been introduced recently to rid the limitation of mucosal vaccine is the DNA vaccine, in which recombinant protein or DNA is used, and which are safer than mucosal vaccines, which are less immunogenic (Ongkudon *et al*. 2011). Thus, challenges that still exist are to develop a proper delivery system or adjuvant to make vaccines more immunogenic.

TARGETING STRATEGIES

To overcome the different levels of challenges of a body's systems, different drug delivery approaches have been adapted. These fall into three categories: (a) Physical approaches; (b) biological approaches; (c) chemical approaches (Rapaka, 1995; Mizuno *et al*. 2003). Physical delivery approaches, such as biodegradable polymers based on nanoparticles, liposomes, osmotic pumps, implants, etc., are to modifying drug pharmacokinetics without essentially affecting the specificity where biological targeting approaches are based on the biological carrier system (bio-conjugation) to cross biological barriers and to ensure site-specific drug release (Misra *et al*. 2003; Bae and Park, 2011). Chemical drug delivery systems (CDSs), another emerging concept in drug delivery systems and initially defined by Bodor and Brewster as chemical compounds produced by synthetic chemical reactions to form covalent bonds between the drug and specifically designed "carrier" and other moieties, include, for example, pro-drugs (Zuo *et al.* 2008; Reichel, 2006; Bae and Park, 2011). The targeting strategy can either be active or passive (Bodor and Brewster, 1991). Sometimes, due to the nature of the challenge and target site, some alternative approaches, like inverse targeting, is an alternative way of passive targeting; alternatively, a number of approaches have been engaged for a single target such as dual or double targeting and combination targeting strategies (Bodor and Brewster, 1991; Zuo *et al.* 2008; Strebhardt and Ullrich, 2008; Patel *et al*. 2010; Bae and Park, 2011).

Active targeting strategy

In active targeting, the delivery system is designed by attaching the drug delivery system – something like an antibody, a carrier protein, or a ligand – allowing it to establish a complex with the target cell where specific receptors are present (Allen, 2002; Mohanraj and Chen, 2006; Bae and Park, 2011; Yoo *et al*. 2011). So, it is obvious that active targeting approaches depend on (1) the specificity of the delivery system and the homing devices where drugs will be attached, for example, antibody, carrier protein albumin, sugar, vitamins, etc. and (2) the capacity of drug delivery from the complex at a specific

receptor site (Allen, 2002; Mohanraj and Chen, 2006; Bae and Park, 2011; Yoo *et al*. 2011). This targeting approach can be further classified into three different levels based on target site: (1) First order targeting (organ compartmentalization) where target sites are the capillary bed of organs or tissues like the lymphatic cavity, peritoneal cavity, cerebral ventricles, lungs, eyes, etc.; (2) Second order targeting (cellular targeting) where target sites are specific cells rather than normal cells like Kupffer cells in the liver, tumor cells, etc.; (3) Third order targeting (intracellular targeting) where target sites are intracellular spaces like the cytoplasm or organelles such as the nucleus, etc. (Charman *et al*. 1999; Santini Jr. *et al*. 2000; Kopecek, 2003). Stimulating the body's immune response against cancer cells by gene therapy (e.g., a DNA cancer vaccine) is a recent area for an active third order targeted drug delivery system (Palmer *et al*. 2002; Bae and Park, 2011). Cancer cell death may be induced by introducing cancer cells with genes encoding apoptosis. This most critical approach for intracellular targeting involves four steps: (1) interaction of the active delivery system with the extracellular plasma membrane receptor; (2) the drug will enter the cell by receptor-mediated endocytosis; (3) fusion of drug with lysosomes; (4) degradation of the homing device and release of the drug or RNA into the intracellular target (Palmer *et al*. 2002; Bae and Park, 2011).

Passive targeting strategy

Simply, passive targeting is an approach where vaccines or therapeutics can escape the body defense mechanism like metabolism, excretion or opsonisation, followed by phagocytosis (Couvreur and Vauthier, 2006; Bae and Park, 2011). So they can easily be circulated throughout the body system and subsequently reach target receptors. These approaches are mainly governed by different characteristics of the delivery system depending on the body's general defensive system (Couvreur and Vauthier, 2006; Bae and Park, 2011). For example, if the delivery system releases a drug having a molecular weight (MW) > 30 kDa, it can avoid quick renal excretion as the MW is ≤ 30 kDa, making it prone to quick renal excretion (Bae and Park, 2011). To do this, formulators normally design drug or vaccine molecules with different types of carrier systems which increase their critical MW (Jain, 2008). However, when increasing the MW, scientists have to consider the size; for example, a > 200 nm size delivery system can blockade the blood capillary (Jain, 2008). In vaccine design, the size is a crucial factor for DC maturation otherwise the vaccine will show lower immunogenic responses (Jain, 2008). The monophasic phagocytic system (MPS) needs a hydrophobic system (treated as a xenobiotic) and opsonisation followed by phagocytosis and subsequent removal from the circulation system into the reticulo-endothelial system for excluding from the body. In this case, scientists should address the hydrophilicity of the delivery system, which that can be achieved in different ways (Jain, 2008). For example, incorporation of a hydrophilicity-imparting agent such as polyethylene glycol (PEG) (Jain, 2008). Surface charge of the delivery system is another important factor which also determines the period to which it can stay and circulate throughout the body's systems (Jain, 2008). As only neutrally charged systems can circulate, they will otherwise be expelled either by Kupffer cells in the liver (for example, a negatively charged system) or by opsonisation (as a positively charged system is recognized by opsonin as a foreign particle) (Moghimi and Patel, 1998). Again, in the case of a vaccine formulation, these types of reorganization could be used in antigen-presenting cells (APCs) to boost immune responses (Moghimi and Patel, 1998; Jain, 2008).

For a non-viral gene delivery system, synthetic vectors for gene delivery must be able to overcome a range of barriers (Jackson *et al*. 2006; Martin and Rice, 2007). These are: (1) stability in the bloodstream; (2) reorganization by receptors of the target cell and subsequent entrance into the cell cytoplasm; (3) if it contains mRNA, cytoplasm will be the end of the journey; if it contains DNA, it will reach the nucleus across the nuclear membrane for transcription (Jackson *et al*. 2006; Martin and Rice, 2007). In passive targeting, all these body defensive challenges can be overcome using a nanocarrier system (Jackson *et al*. 2006; Martin and Rice, 2007). As DNA is a polyanion and can bind with polycations such as poly(L-lysine) or poly(ethylene imine), it can make polyplexes which are approximately 100 nm in size and discreet in nature. This size allows it to penetrate through small pores of the membrane (Jackson *et al*. 2006; Martin and Rice, 2007; Jain, 2008). This can be protected by surface coating with a steric stabilizer like PEG followed by a multivalent hydrophilic lateral stabilizer such as poly[N-(2-hydroxypropyl) methacrylamine] (pHPMA) which makes it a stealth vector for escaping the body's defense system (Jackson *et al*. 2006; Martin and Rice, 2007). This will be necessary for receptor-mediated uptake of these stabilized polyplexes by receptor-positive cells, using transferrin, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) as targeting ligands. Following receptor-mediated uptake into cells, DNA polyplexes are usually found in the endosomes (Kircheis *et al*. 2001). Their entry from the endosome into the cytoplasm can be mediated in various ways like the peptide melittin or pH-responsive peptides, etc. (Kircheis *et al*. 2001). In the cytoplasm, polymer coating becomes destabilized by reducing the disulphide bonds and releasing DNA for cytoplasmic expression (Kircheis *et al*. 2001). However, entry of exogenous DNA into the nucleus is extremely difficult, particularly in non-dividing cells, where the nuclear membrane provides a persistent barrier to entry (Kircheis *et al*. 2001; Jackson *et al*. 2006; Martin and Rice, 2007). For nucleus entry, DNA should be designed especially with a nuclear localization sequence (NLS) which ensures efficient nuclear uptake and subsequent nuclear expression (Lam and Dean, 2010). An alternative approach to enhanced nuclear uptake is based on the mechanisms employed by an adenovirus, where the adenovirus hexon protein mediates efficient transcytoplasmic transfer of the virion, delivering it to the nuclear pore complex for expression (Lam and Dean, 2010).

In the passive targeting interventions, for example pathophysiological approaches, if we wish to target tumor cells in the liver for a chemical therapeutic or a DNA vaccine (e.g., an anti TGF-β DNA vaccine), we can formulate it to be coated with a proper carrier system having a proper size and charge but hydrophobic in nature (Kresina, 2001; Lam and Dean, 2010). After administration, it will be euphonized and sent to the liver cells but cannot be destroyed due to its coating system until it reaches the tumor cells in the liver as the inner temperature of tumor cells will be higher than normal liver cells (Kresina, 2001). Again, as an example of a physicochemical approach, if a drug is highly soluble and excreted out quickly, MW enhancement (>30 kDa) is a rapid approach to avoid renal excretion and give more time to reach the target site (Kresina, 2001).

Other targeting strategies

Inverse targeting strategy

It is sometimes possible to escape the body's defense system by an alternative way of passive targeting approaches, also known as the inverse targeting approach (Paasonen, 2010). For example, the rapid uptake of a colloidal carrier system by the

reticuloendothelial system (RES) can be escaped by pre-treatment of blank colloidal carriers or macromolecules like dextran sulphate to saturate the RES rather than passive targeting approaches that involve an increment in molecular size (Paasonen, 2010). This approach leads to a RES blockade and consequent impairment of the host defense system (Paasonen, 2010).

Dual targeting strategy

The dual targeting approach is an efficient approach against virus infection and its drug resistance where the carrier system used to load the antiviral drug has an synergistic effect on the action of the drug (Kircheis *et al*. 2001; Martin and Rice, 2007; Jain, 2008; Bae and Park, 2011). Based on this approach, bioconjugates can be prepared using different types of natural and synthetic molecules such as antibodies, immunetoxins, CD4, glycoprotein, etc. (Kircheis *et al*. 2001; Martin and Rice, 2007; Jain, 2008; Bae and Park, 2011). Conjugation can be achieved using various non-covalent and covalent techniques. These dual targeting approaches can also be implemented for an enzyme immune assay system (e.g., antibody-enzyme conjugates), vaccine research and antibody production (e.g., Hepten-carrier conjugates), etc. These become important research issues especially for non-viral gene delivery systems and cancer vaccines (Kircheis *et al*. 2001; Martin and Rice, 2007; Jain, 2008; Bae and Park, 2011).

Double targeting strategy

This new concept of drug delivery is designed to improve the drug therapeutic index in terms of selectivity and control release (Kircheis *et al*. 2001; Martin and Rice, 2007; Jain, 2008; Bae and Park, 2011). Selectivity to a target site is ensured by linking with antibodies that are specific either to a particular antigen or cell lines expressing a cell-specific receptor. Controlling the release of a drug depends on the device or system which will carry the drug and can be stimulated or activated spatially after reaching the target site which is solely governed by the linked antibody's specificity (Kircheis *et al*. 2001; Martin and Rice, 2007; Jain, 2008; Bae and Park, 2011). Therefore, the system will contain a drug loader that can be controlled spatially and a linked antibody to govern its selection to specific molecular sites (Kircheis *et al*. 2001; Martin and Rice, 2007; Jain, 2008; Bae and Park, 2011).

Combination targeting strategy

The combination targeting approach is an upgraded strategy for the delivery of site-specific proteins and peptides. It is equipped with carriers, polymers and homing devices of molecular specificity that could provide a direct approach to the target site (Roth *et al*. 2008). This approach is being adapted to avoid a number of shortcomings of proteins and peptide delivery techniques (Roth *et al*. 2008). These are: (1) permeability problem of large peptides; (2) chemical decomposition of homing devices; (3) non-specific drug release due to target tissue heterogenecity; (4) immune response against the system, etc. (Roth *et al*. 2008). The problem of permeability can be solved by modification of peptides using natural polymers such as PEG. A pro-drug is another strategy for avoiding the chemical decomposition of homing devices (Roth *et al*. 2008). For site-specific drug release, scientists can employ either different types of carrier systems or spatially controlled devices (Roth *et al*. 2008).

CARRIER-BASED PHYSICAL DELIVERY APPROACHES

Nanoparticles

In the field of controlled and targeted drug delivery system, biodegradable and biocompatible polymers based on colloidal carriers are recently playing a governing role in research and development (Kircheis *et al*. 2001; Martin and Rice, 2007; Jain, 2008; Bae and Park, 2011). The concept of a

nanoparticle, among them, is the most prominent branch of study that can be used for site-specific delivery as well as for controlling the release from burst to pulsatile approaches (Petros and DeSimone, 2010; Bae and Park, 2011; Wani *et al*. 2011). Nanoparticles, are sub-nanosized colloidal structures, generally composed of natural, synthetic or semi-synthetic biodegradable and biocompatible polymers such as gelatin, albumin, poly(lactic acid), poly(lactic glycolic acid), etc. (Petros and DeSimone, 2010; Bae and Park, 2011; Wani *et al*. 2011). Among the available natural hydrophilic polymers such as gelatin, albumin, etc., most of them suffer a number of disadvantages: (1) lack of uniformity; (2) chance of biodegradation before reaching the target site due to less physical stability; (3) antigenic response, etc. (Bae and Park, 2011). Drugs can either be entrapped into the reservoir or the matrix system or be absorbed onto the surface of these particulate systems (Petros and DeSimone, 2010; Bae and Park, 2011; Wani *et al*. 2011).

Like all colloidal drug carrier systems, the bio-distribution and bio-fate of nanoparticles can be described as rapid opsonization and followed by excretion by the RES macrophages (Bae and Park, 2011). About 90% intravenously injected nanoparticles are absorbed by the liver and spleen within minutes (Petros and DeSimone, 2010). This unique feature of nanoparticles has been engaged for nanoparticles based on passive targeting chemotherapy of RES-localized tumors in the liver where a drug will reach rapidly and the release of that drug, from burst to controlled pattern, will depend on the polymeric characteristics of those carrier nanoparticles (Petros and DeSimone, 2010). For tissue targeting other than RES requires the escape from rapid absorption into the liver RES. This can be achieved by increasing particle size (< 100 nm) and hydrophilicity of the nanoparticles (Petros and DeSimone, 2010; Wani *et al*. 2011). The process of escaping RES absorption has already been discussed in passive targeting approaches. Nanoparticles coated with polysorbate open the way of greater transportation of a drug into the brain across the BBB (Petros and DeSimone, 2010). Polysorbate-loaded nanoparticles link with apolipoprotein E of blood plasma which in turn interact with LDL (low density lipoprotein) receptors on endothelial cells in the brain capillary and lead to their cellular uptake through the BBB. Sub-cutaneous nanoparticle injection is a useful tool for chemotherapy against lymphatic tumors or metastasis (Petros and DeSimone, 2010; Wani *et al*. 2011).

Surface modification of nanoparticles offer numerous opportunities for drug targeting as it allows the specific bio-chemical interaction between the surface of nanoparticles and the proteins or receptors expressed on target cells (Petros and DeSimone, 2010; Wani *et al*. 2011). Beside this, surface modification or engineering can be employed to prepare stealth nanoparticles through steric stabilization to deter the opsonization process, or prepare site-specific antibody-coated nanoparticles, magnetically guided nanoparticles and bioadhesive nanoparticles (Petros and DeSimone, 2010; Wani *et al*. 2011).

The liposome has tremendous potential as the drug delivery system for both passive and active targeting approaches due to its enormous structural diversity as well as composition possibilities (Cao and Suresh, 1998). Simply, liposomes are organized phospholipid vesicles that have been used to encapsulate protein and DNA (Ratnam *et al*. 2006). Lipid composition makes them unique for rapid interaction with microphages and DCs via cell surface receptors, such as CD1a, after complement activation (Cao and Suresh, 1998; Ratnam *et al*. 2006). It allows them to escape the challenges imposed by the body's

defense system. Passive approaches that target macrophages can be used to deliver immunomodulators, as well as cytotoxic and anti-microbial agents (Ratnam *et al*. 2006). Liposomes can also couple to an anti-transferrin receptor antibody at the BBB and easily cross through endocytosis (Ratnam *et al*. 2006). Multi-drug resistance (MDR), which is another tremendous challenge for formulation scientists, can be overcome by a liposomal delivery system (Mecke *et al.* 2006). Most of MRD is related with the over expression of some drug efflux pumps, known as P-glycoprotein pumps (PGPs) and Multi-drug resistance associated protein pumps (MRPs) as these pumps efflux and reject positively charged amphipathic drugs (mostly anticancer drugs) from cells (Mecke *et al.* 2006; Ratnam *et al*. 2006). This action may cause less intracellular drug accumulation which leads to drug resistance. A negatively charged liposomal system can easily avoid the efflux process of the MRP (Mecke *et al.* 2006; Ratnam *et al*. 2006). Engineered liposomes and lipid complexes such as pH-sensitive liposomes, immunoliposomes, cationic liposomes (lipoplexes), fusogenic liposomes, genosomes, etc., which show much potential in gene delivery, have a clear advantage of high transfection efficiency over other non-viral vector systems (Mecke *et al.* 2006; Ratnam *et al*. 2006). pH-sensitive liposomes undergo slight destabilization in the mild acidic medium of endosomes after endocytosis and cause the selective release of mRNA into the cytoplasm followed by subsequent translation. Immunoliposomes can absorb DNA on their surface and transfer it into the cytoplasm without allowing lysosomal destruction (Cao and Suresh, 1998; Mecke *et al.* 2006; Ratnam *et al*. 2006). Genosomes, a complex formulation of DNA with various cationic liposomes (lipoplex), is a colloidal suspension that can transfer DNA from the site of injection to the target cell surface though biological fluid such as serum and release it into the nucleus of target cells for DNA expression (Mecke *et al.* 2006; Ratnam *et al*. 2006). Liposomes can be classified based on composition, its applications or diameters (Cao and Suresh, 1998; Mecke *et al.* 2006; Ratnam *et al*. 2006).

Traditional bilayer and multilayer liposomes are physically unstable and rapidly leak the encapsulated material (Mecke *et al.* 2006; Ratnam *et al*. 2006). This problem can be solved by using the concept of polymerized liposome nanoparticles which can maintain their size and integrity even upon oral administration. Nanoparticle-based vaccines can carry multivalent surface antigens like liposomes, sugar, protein, etc. which elicit a significant increase in the immune response (Mecke *et al.* 2006; Ratnam *et al*. 2006).

BIOCONJUGATES AS BIOLOGICAL DELIVERY APPROACH

Bioconjugation, a biological drug targeting approach, involves the covalent or non-covalent linkage of two or more molecules to form a novel complex having combined properties of each of them to deliver the drug to the specific target site by avoiding enzymatic degradation pathways, rapid RES uptake and other challenges (Mohanraj and Chen, 2006). Examples of bio-conjugates include several vaccines, pegylated proteins and antibody-linked molecules. The goal of bioconjugates depends mainly on the nature of biocarriers (Mohanraj and Chen, 2006). Highly investigated biocarriers can be categorized into: (I) Macromolecules – for example, antibody, immunotoxins, oligonucleotides, CD4, interleukins, interferons, transferrins, insulin, etc.; (II) Enzymes; (III) Glycoproteins (Mohanraj and Chen, 2006). The most common types of bioconjugates in localized drug targeting approaches vary from simple types of bioconjugates like small molecule (such as biotin) to protein bioconjugates, protein-protein bioconjugates (such as the coupling of an antibody to an enzyme), to very complex types such as oligosaccharides,

nucleic acids, synthetic polymers (such as PEG, poly-L-lysine) and carbon nanotube bioconjugates, etc. (Mohanraj and Chen, 2006).

PRO-DRUG, AS CHEMICAL DELIVERY APPROACH

The term "pro-drug" was first introduced in 1958 to describe compounds that undergo biotransformation prior to their therapeutic activity (Rautio *et al.* 2008). It is a chemically modified inactive drug moiety which will be converted into an active one by enzymatic action after reaching specific target sites (Rautio *et al.* 2008). By applying pro-drug technology, an example of a chemical targeting approach, the clinical usefulness of a drug molecule may be enhanced without modifying the pharmacological activity of the parent drug. In addition, there should be considerable knowledge on a particular enzyme system and its molecular and functional characteristics upon which the release of the drug after appropriate cleavage from the drug carrier depend (Rautio *et al.* 2008).

Oral drug absorption, which is rate limiting for poorly soluble and poorly permeable drugs, can be enhanced by using gastrointestinal enzymes that target a pro-drug based chemical delivery system (Rautio *et al.* 2008). For colon-specific drug delivery, the glycosidase activity of colonic microflora can be used by chemically modifying the drug molecule into its glycoside derivatives (Rautio *et al.* 2008). This pro-drug is hydrophilic in nature and is not absorbed by the intestine but when it reaches the colon, it can be effectively cleaved by bacterial glycosidases of the microflora to release and be absorbed by colonic mucosa (Rautio *et al.* 2008). The delivery of peptides to the brain is challenging due to their hydrophilic nature and their rapid degradation by peptidases localized within the capillary endothelium. There is promise in transferring ester-linked peptide pro-drugs through the BBB without peptidase activity (Rautio *et al.* 2008). Glycin pro-drugs such as Milacemide (2-*n*-pentylaminoacetamide) are another example of a pro-drug-based brain targeting approach where Monoamine oxidase (MAO), a mitochondrial enzyme which catalyzes the oxidative deamination of amines, plays a vital role in the cleavage of the glycin linkage from the active drug after crossing the BBB (Balant *et al*. 1990; Rautio *et al.* 2008). Glycin, which is incorporated chemically as a glycinamide linkage with bioactive peptides, enhances its lipophilicity to ensure its transportation across the BBB (Balant *et al*. 1990; Rautio *et al.* 2008). Beside this lipid-mediated transportation, drug molecules can be targeted to the central nervous system (CNS) based on their interaction with endogenous transport systems located within the brain capillary endothelium or the neuroepithelial cells of the choroid plexus (Balant *et al*. 1990; Rautio *et al.* 2008). These endogenous transport systems are: (a) carrier-mediated transport (CMT), (b) active efflux transport (AET) and (c) receptor-mediated transport (RMT). Among them, CMT systems that show structure-specific (stereospecific) transportation mainly transport nutrients, vitamins or hormones into the CNS (Balant *et al*. 1990; Rautio *et al.* 2008). Pro-drug chemical approaches through either (i) the modification of drug into a "pseudonutrient" structure or (ii) the conjugation of a drug with a nutrient shows promise in CMT systems for neuroactive molecules. L-DOPA is the first example of a CMT-targeted, pro-drug-based delivery system (Balant *et al*. 1990; Rautio *et al*. 2008).

CONCLUSION

Different types of drug delivery strategies that are engaged to overcome the barrier systems for drug targeting have been systematically described in brief. The selection of suitable strategies, which is a critical step, depends on the characteristics of

physiological barriers and available tools and techniques. Drug targeting is clearly not only a challenging field in pharmaceutical sciences and medicine but also a fascinating field for innovation as it is now not limited to traditional concepts rather than the combination concepts of biology, chemistry, physics and engineering.

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