REVIEW ARTICLE

Accepted 1 November 2004
Revised 1 June 2004
Received 2 May 2003
Indian J. Pharm. Sci., 2004, 66(6): 711- 720

Novel Approaches for Drug Delivery to the Brain

V. SONI, M. K. CHOURASIA, Y. GUPTA, A. JAIN, D. V. KOHLI AND S. K. JAIN*
Pharmaceutics Research Projects Laboratory,
Department of Pharmaceutical Sciences,
Dr. Hari Singh Gour Vishwavidyalaya, Sagar-470 003

The major obstacle in the development of centrally active therapeutics is the lipoidal blood-brain barrier. While a variety of systems to transport compounds into the central nervous system have been designed. The current challenge is to develop drug delivery strategies that will allow the passage of drug molecules through the blood-brain barrier in a safe and effective manner. The invasive approach involves the blood-brain barrier disruption either by osmotically or by using biologically active agents. The above strategies are primarily aimed at short-term application in the treatment regimens of malignant brain tumours while chronic degenerative disorders will require long term application of the therapeutic agent. Owing to this fact non-invasive approach for drug delivery to brain via the systemic route have been developed. Physiological strategy has been to conjugate the therapeutic drug with a protein or a vector or a monoclonal antibody that gains access to the brain by receptor mediated transcytosis. The uses of the drug delivery devices such as liposomes and nanoparticles enhance the penetration of several drugs across the brain. This review will provide an insight into some of the strategies developed to enhance drug delivery across the blood-brain barrier.

The brain is a delicate organ with many vital functions and many formidable mechanisms isolate and protect it from the outside world. Unfortunately, the same mechanisms that prevent intrusive environmental chemicals accessing the brain also prevent the access of therapeutic chemicals. Now, it is well established that the brain is tightly segregated from the circulating blood by a unique membranous barrier the blood brain barrier (BBB)[1,2]. The BBB represents a formidable obstacle for a large number of drugs, including the majority of anticancer agents, peptides and nucleic acid. As a consequence this barrier prevents effective treatment of many severe and life threatening disease like brain cancer. The BBB is a tight junction of brain capillary endothelial cells (BCECs), which abolish aqueous paracellular pathways across the cerebral endothelium, and thus prevent the free diffusion of solute into the brain. Therefore, the diffusion or permeability is dependent on the lipophilicity of drug and solutes. It is also seen that some drugs like vincristine, vinblastine and cyclosporin A are highly lipophilic but the permeation is quite slow[1-4]. This could be due to the existence of multiple mechanism of drug transport through the BBB as well as passive diffusion. Endothelial cells also contain many mitochondria-metabolically active organelles and active transport can significantly alter both inward and outward transport of the compounds that are substrates of the corresponding transporters. Overall, the BBB is highly efficient and makes the brain practically inaccessible to lipid-insoluble compounds. Brain delivery of such compounds therefore requires a strategy to overcome the BBB.

Due to the diversity in neuropharmaceutical as well the transport mechanism, the drug delivery strategies may be broadly classified into categories local versus systemic,

*For correspondence
E-mail: drskjainin@yahoo.com

invasive versus non-invasive and pharmacological versus physiological, as shown in fig.1.

INVASIVE STRATEGIES

Drug delivery based on the BBB disruption:

Temporary physicochemical disruption of endothelial integrity of the brain is one of the invasive strategies for drug delivery to the brain. Hypertonic disruption with help of 25% mannitol or arabinose enhances the delivery of the small molecular weight cytostatic agent to the brain tumours[5]. The underlying mechanism is a sequence of endothelial cell shrinkage, disruption of tight junctions and vasodilation by osmotic shift. Morphological study like light microscopy and electron microscopy provide the evidence of brain uptake of macromolecule and ultrastructual changes such as swelling of astrocytic processes and severe mitochondrial damage of neuron, respectively[6,7]. In addition to opening of junctional complexes and the formation of interendothelial gaps, transendothelial opening and tracer passage through the cytoplasm of injured endothelial cells were observed. In response to hypertonic barrier disruption, there was also evidence of prolonged cellular stress or injury in neurons and glia, as expressed by the induction of heat shock protein[8]. The osmotic disruption has been tested as a strategy for the brain delivery of macromolecular drugs such as monoclonal antibodies, nanoparticle and viruses[9-11].

BBB opening also achieved by the receptor mediated mechanisms. Vasoactive compounds like prostaglandin, histamine, serotonin, leukotriene C4 (LTC4) and bradykinin have all been shown to induce BBB leakage[12]. The effects of LTC4 and bradykinin are more pronounced on the blood tumour barrier than on the normal BBB. This is due to the endothelial expression of γ-glutamyl transferase (γ-GT) enzyme which metabolitzes and inactivates LTC4 to LTD4[13] while tumour blood vessels are unable to express equivalent activity of γ-GT. This difference has been exploited for selective opening of the tumour barrier by intercarotid administration of LTC4. On the other hand, bradykinin opens the barrier in the high molecular weight range, too. It acts on endothelial cells through B_2 receptors located on the abluminal side. Normal brain tissue is protected from varied opening by bradykinin in the vascular lumen because the peptide cannot access these receptors. In tumour vessels the barrier integrity is sufficiently compromised to allow for a bradykinin-mediated additional opening at low peptide concentrations[14].

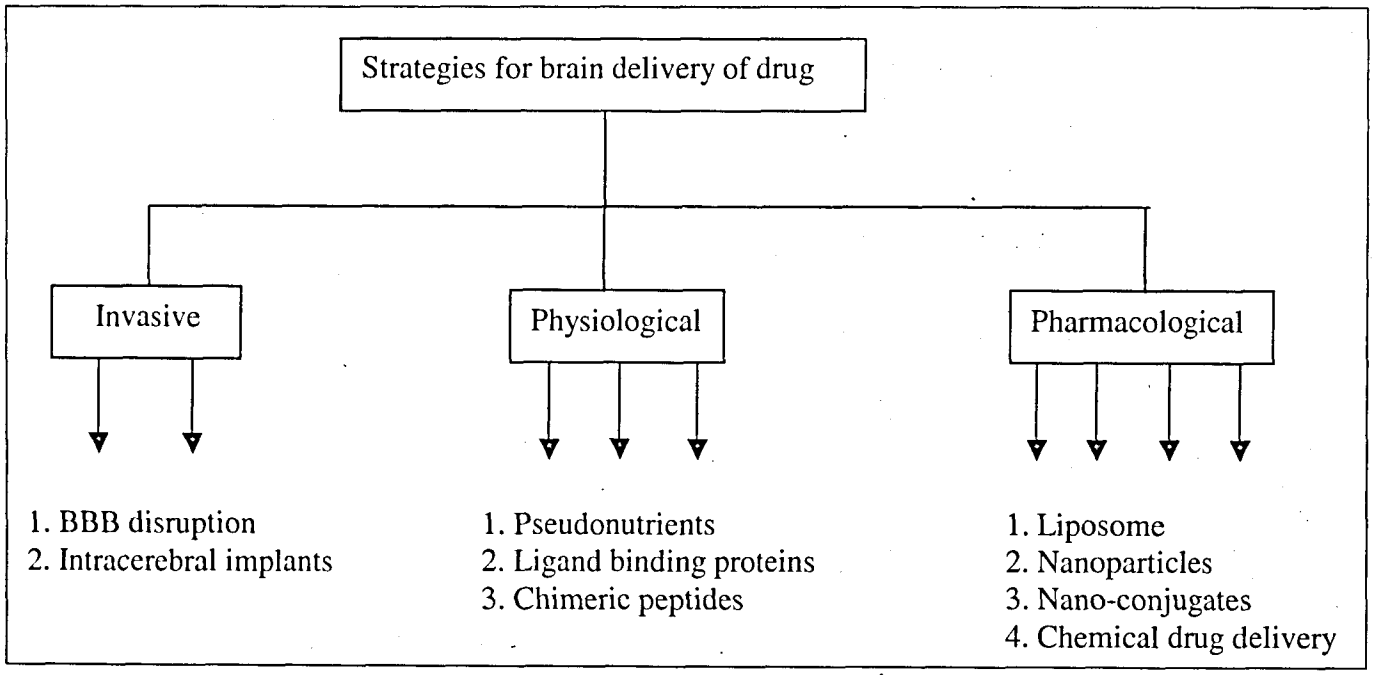

Fig. 1: Various strategies for trageted drug delivery to brain

Variou strategies for drug delivery to brain include invasive approach, physiological approach and pharmacological approach

Intracerebral implants:

Intracerebral chemotherapeutic delivery by polymeric implants increases survival of humans with recurrent malignant gliomas[15,16] and of animals with transplanted gliomas[17-24]. Drug added to polymer pellet implants intracranically bypass the BBB and release drug molecules locally in the brain in a sustained fashion. Malignant gliomas are deeply in the brain[25-27] and thus the effectiveness of the drug delivered by polymers is dependent on whether drug molecule can be transported a sufficient distance from the implanted site to reach malignant gliomas. Existing knowledge regarding the local distribution and pharmacokinetics of anticancer drugs delivered by polymer implants is largely based on studies in rodents[20,28-32]. To improve the pharmacokinetic basis of intracerebral polymeric chemotherapeutics in human, biodegradable polyanhydride pellets containing carmustine [1,3-bis (2-chloroethyl)-nitrosourea] or 4-hydroperoxycyclophosphamide or paclitaxel were implanted into the brain of cynomolgus monkeys. It was found that drug concentration in cerebral blood and cerebrospinal fluid (CSF) during the subsequent 30 days period was extremely high in the vicinity of the implantation site. It was also found that lower concentrations of the drug could be maintained at distant locations in the brain for a prolonged period after a single dose of polymer-encapsulated drug. High concentration near the polymer pellets can prevent local tumour recurrence. The prolonged low concentration of drug is effective in treating multifocal gliomas and tumours that reoccur at sites distant from the primary tumour[33]. The polymer implants substances designed to treat tumour can be administered directly to the affected areas of the brain and release in a sustained manner for a long period of time. This strategy is the most direct way of increasing the focus of a therapeutic agent that is already targeted.

PHYSIOLOGICAL STRATEGIES

The above strategies are primarily aimed at short-term application in the treatment regimens of malignant brain tumours while chronic degenerative disorders will require long term application of the therapeutic agent. Owing to this fact, non-invasive approach for drug delivery to brain via the systemic route have been developed.

Pseudonutrient approach:

Peptide drug design may incorporate a specific molecular characteristic that facilitates the drug to be transported by one or more of the inwardly directed nutrient carriers. The BBB expresses several transport systems for nutrients and endogenous compounds[34,35]. Utilization of these transport systems is a potential strategy for controlling the delivery of drugs into the brain. These drugs must have a molecular structure that mimic the endogenous nutrient[36]. Biphalin, a potent opioid analgesic[37,38], is a peptide drug that has been shown to use the neutral amino acid carrier system to gain across the brain[39]. Neutral amino acid carrier, which is a high capacity one, is more conducive for drug transport. Chemical groups could be designed with the ability to attach to specific drugs rendering them substrates for carriers or drugs specifically designed for a carrier mechanism. The hexose and large neutral amino acid carrier have the highest capacity and are best-suited option for the delivery of substrates to the brain[40].

Ligand binding proteins:

Protein ligands possess various properties like high affinity to receptors and selectivity for targeting, which increase the interest towards the use of proteins as a delivery tool for targeting drugs to the brain. Various systems have now been developed that incorporate proteins as the central ligand–binding component such as lectins used as a ligand binding protein for brain targeting of glucose–triggered glycosylated insulin and bispecific antibodies[41]. Other ligand binding protein classes include biotin-binding proteins, lipid-binding proteins and avidin binding proteins. Avidin is a basic tetrameric glycoprotein isolated from egg white and streptavidin secreted by *Streptomyces avidinii*. The biotinylation of drug can be obtained by linking with disulfide (–S-S-) bond under mild conditions[42]. Although the avidin biotin complex is highly stable, the biotinylated drug can easily be released at the target site[43]. Antibody–avidin, cationized albumen- avidin fusion protein and more complicated avidin containing system have been used for the successful delivery of biotin, biotinylated bioactive peptide, biotinylated nucleic acid and other molecules to the brain as well as to cancer cells[43-45].

Cationized albumin appears to be useful for the delivery of the active agents across the BBB to the brain. The active agents attach covalently or bound to a cationized albumin (neutral)-avidin conjugates[46]. The main advantages of the cationized albumin are compatibility with the human blood, plasma protein, and body components. Like avidin biotin conjugates immunoglobins occupy a special place in the field of ligand binding proteins because of their ability

to collectively recognize an almost infinite number of ligand molecules. The effective immunocomponents are vaccines, antibodies, monoclonal antibodies, and immunotoxins. Antibody molecules are capable of both, incredible diversity and high specificity. Individually, they may bind one or only a few compounds with high affinity but collectively they are able to recognize virtually any molecule. As such, they offer an almost unlimited versatility[47,48].

Hybridoma technique is widely used for the production of monoclonal antibodies. This technique is quite adoptable and produces highly specific carrier molecule. Temperature, pH, or protease sensitive monoclonal antibodies are the stable, specifically designed and modified systems. A new trend in the development of antibody-antigen based targeting is the use of antibody fragments are instead of whole immunoglobulin molecule. Now recombinant antibody fragments are a well-sought strategy for the development of antitumor drug delivery systems. They can easily be incorporated into the fusion proteins (carcinoembryonic antigen single chain Fv protein, Sc Fv along with therapeutic entities. This phenomenon is exploited for the delivery of antitumor drugs to the brain and in tumour imaging studies. But, their accumulation at target site slightly decreases due to monovalent antigenic nature of ScFv, which allows for their rapid removal from circulation and tumour accumulation. Another promising approach is two-step cancer therapy. In this method, monoclonal antibody-enzyme conjugates are utilized for the activation of the anticancer prodrugs[49-51]. This is a two-step approach to drug delivery in which a mAb–enzyme conjugate that specifically localized into solid tumour masses is administrated followed by systemic treatment with an anticancer prodrug. Upon contact with targeted enzymes, the prodrug is converted into an active cytotoxic drug. The advantage of two-step targeting strategy over the use of covalently linked mAb–drug conjugates[52] for selective drug delivery is that a single localized mAb–enzyme conjugate is capable of catalytically generating large amount of active drug and the drug thus formed can penetrate into regions of the tumour mass that are inaccessible to the conjugate resulting in high intratumoral drug concentrations[53] and pronounced antitumour activities[51,54].

Chimeric peptides:

Synthesized chimeric peptides are another possibility for the drug delivery to the brain[55]. Chimeric peptides are generated by linking of a drug (that lacks transport at BBB) to a vector at the luminal membrane of brain capillary endothelial cells, which initiates receptor-mediated or adsorption-mediated transcytosis. The mode of delivery is schematically shown in fig. 2. Vectors are the structurally diverse compounds like insulin and insulin like growth factors (IGF-I and II)[56] transferrin[57], low-density lipoprotein[58] and leptin[59]. The qualities of the chimeric peptides is dependent on each of its domain i.e. vector, linker and drug.

The vector moieties provide targeting and transport of drug to brain endothelium and beyond. Drugs could be designed to act at the level of the BBB to a target in an extracellular space or on brain cells. The concepts of the chimeric peptides in a three-dimension arrangement are given in fig. 3. The overall process is designated as transcytosis and is composed of binding to a luminal plasma membrane receptor, endocytosis, transfer through the endothelial cytoplasm, to the abluminal side and abluminal exocytosis into brain interstitial space. Chimeric peptides are required to be stable in the circulation before brain uptake occurs and either amide bonds, thioether, or disulfide linkage are suitable in terms of stability in plasma compartment[60]. Insulin, IGF, leptin and transferrin are some of the receptors, which are present at the BBB and their relative affinities, is proportional to the respective dissociation constant as summarizing in Table 1[61].

The use of such vector may display undesirable pharmacological activity for examples, when insulin is used as a vector it could cause hypoglycaemia. Also, the use of a vector whose biodistribution and intracellular routing differ from the intended site of drug delivery may deliver the drug to inappropriate sites, such as when an insulin receptor monoclonal antibodies is used as a vector it will deliver the drug to the brain as well as peripheral tissue which could be due to the presence of insulin receptor in peripheral tissue and brain[62]. While in case of transferrin used as a vector, it compete with the presence of endogenous transferrin at the BBB transferrin binding sites. The use of IGF-I and IGF-II as transport vector is difficult because of the very avid binding (>99%) of IGF by specific plasma binding proteins for these particular growth factors. An alternative approach however utilized vectors based on monoclonal antibodies specific to the extracellular domain of a peptide or protein receptor at the BBB. Such vectors fulfil the criteria of binding to the receptors at a site distinct from the ligand binding site and not interfering with the endocytosis process[63]. A model vector for receptor-mediated transcytosis has been the OX - 26 murine monoclonal antibodies, which recognize an external epitope of transferrin receptor. Avidin-biotin conjugated vectors are

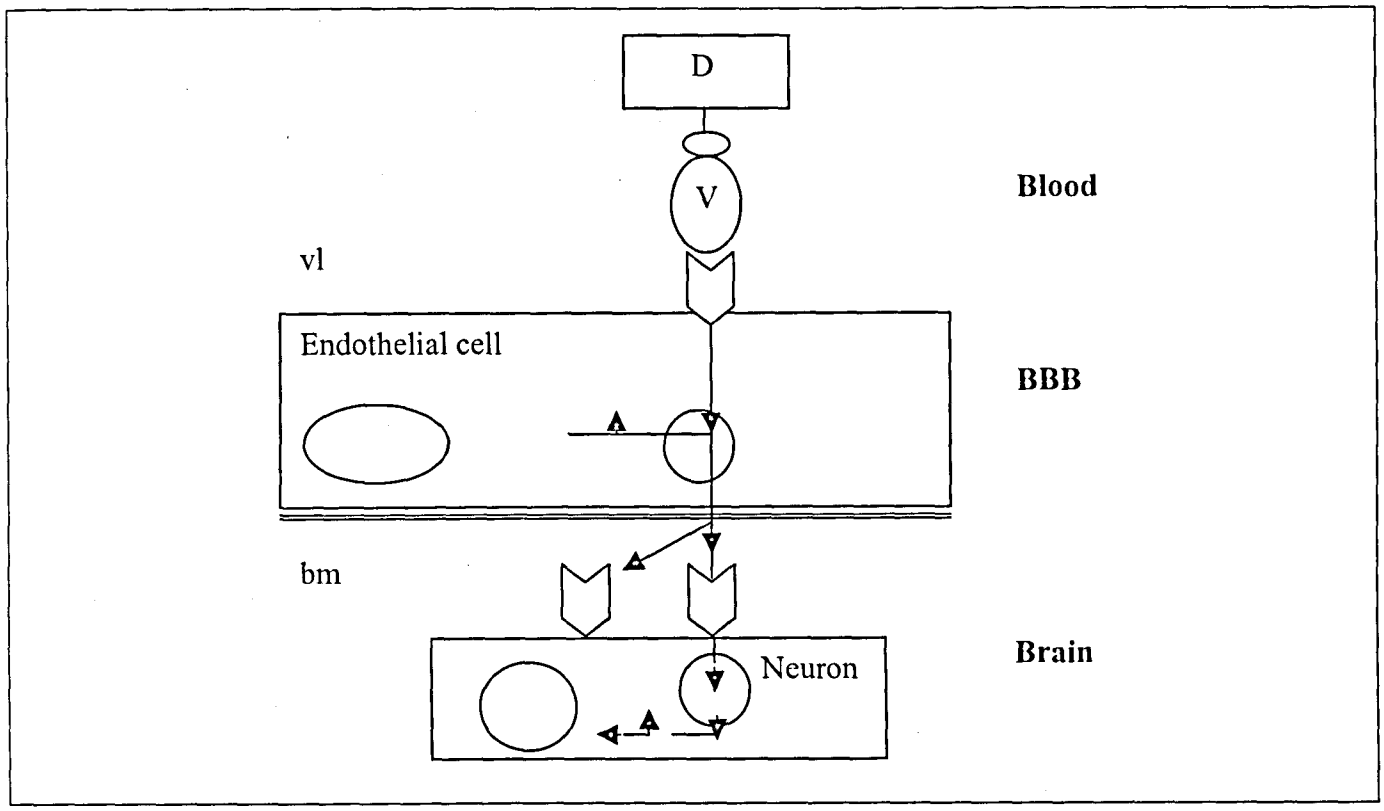

Fig. 2: Mode of drug delivery through transcytosis.

The receptor on the vesicle lumen (vl) binds the vector (v) moiety conjugated with drug (D) and transports into the brain through endocytosis.

also used for the transfer of monoclonal antibodies coupled drug to the BBB[64].The binding of monoclonal antibodies with transferrin receptor enable to penetrate it to the BBB, and also utilize to transport various neuropharmaceuticals[65].

Antisense oligodeoxynucleoides (ODNs) are another class of highly hydrophilic macromolecular drugs that require transcellular delivery. They are potential neuropharmaceuticals with high degree of specificity and react in sequence specific mechanism with target m RNA molecula in the cytosol. Because ODNs have intracellular sites of action in the cytoplasm or nucleus they require yet another transmembrane transport beyond their delivery through the BBB[66].

PHARMACOLOGICAL STRATEGIES

Liposomes:

Liposome are lipid vesicles first characterized by Bangham[67]. Liposomes were initially developed as models of biological membranes. Their potential as a drug delivery system was recognized only over recent years. Basically, liposomes are well-defined lipid vesicles that offer an immense advantage of targeting the drug to selected tissues via appropriate modifications mediated by either passive or active mechanisms. Liposomes with mean diameter less than 100 nm selectively extravasate in tissues characterized by a leaky vasculature (eg. solid tumours) and exhibit tumour targeting[68].

Liposomes are biocompatible, non-toxic and biodegradable carrier constructs, which offer the possibility of carrying hydrophobic, hydrophilic or amphoteric molecules. They can act as carrier for drugs[69], enzymes[70], proteins[71], anticancer substances[72] and other macromolecules[73]. Liver, spleen and other tissue including the brain can accept parentally injected liposomes[72 74 75].

Liposomes can modify the therapeutic profile of selected antitumor drugs in a very favourable manner by reducing drug toxicity to critical host tissues[76 77]. These improvements can be achieved through physical means by

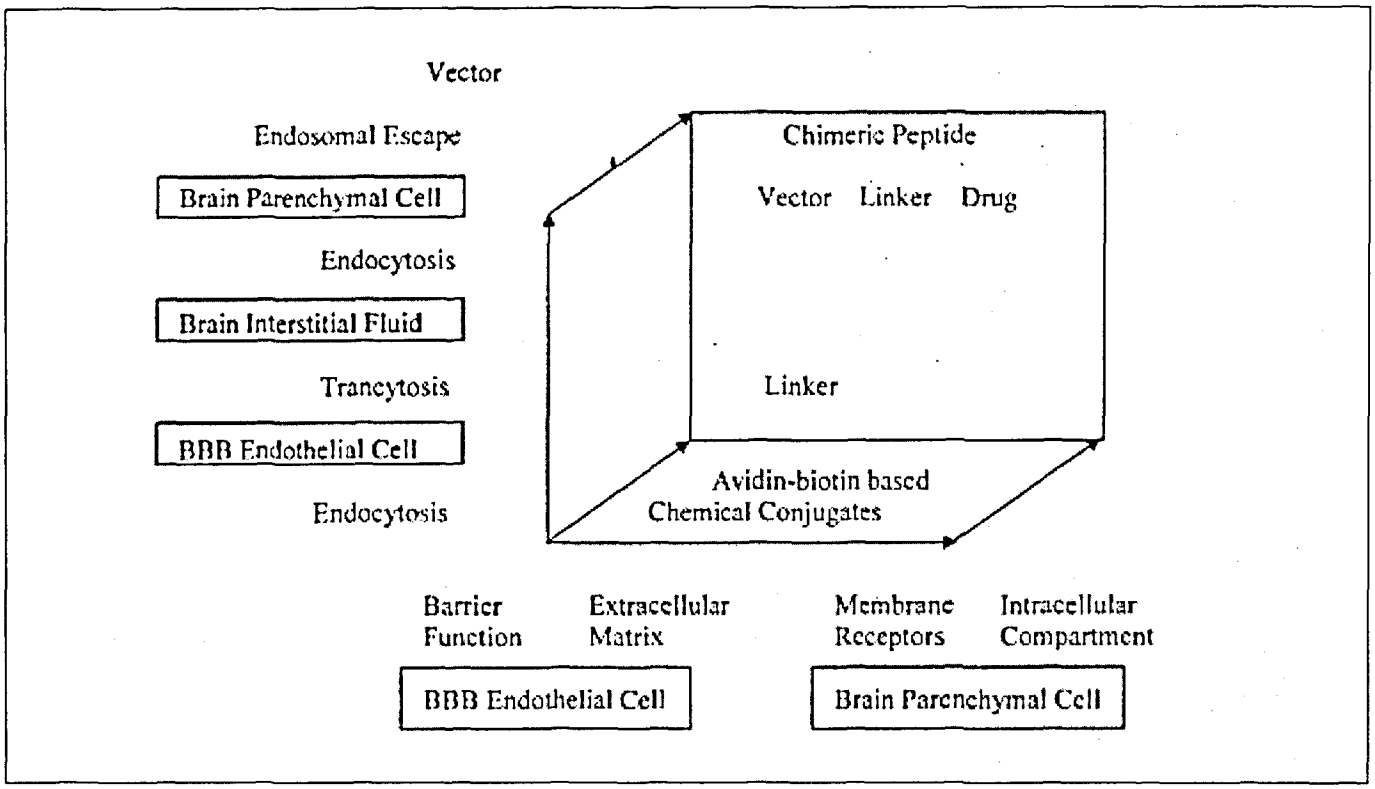

Fig. 3: Chimeric peptides concepts in a three dimensional arrangements to give an impression of its multiple variations.

retaining drug within vesicles while in the circulation thus avoiding/minimizing uptake by sensitive normal tissue and in addition by selectively extravasating into target tissue releasing active drug.

Liposome based anticancer chemotherapy offers the advantage of reduced systemic toxicity combined with selective drug delivery into tumour[78]. Recent studies revealed that a new formulation of small sized (less than 100 nm), long circulating liposome (stealth/sterically stabilized) appears to offer selective tumour localization[79]. Prolonged circulation of liposomes is achieved by insertion of gangliosides or PEG derivatized lipids within the bilayer of conventional liposomes.

TABLE 1: THE RELATIVE AFFINITIES OF BBB TO THE VECTORS

Peptide	Dissociation constant (nM)
Insulin	1.2±0.5
IGF-I	2.1±0.4
IGF-II	1.1±0.1
Transferrin	5.6±1.4
Leptin	5.1±2.8

This localization is probably related to long circulation time of liposome and to increase probability for extravagation to the tumour vascular endothelium[80]. It has also been demonstrated that the selective tumour localization of doxorubicin encapsulated in stealth liposomes (SLs) is associated with superior therapeutic activity over free drug activity in various systemic models[79-82]. This characteristic makes SLs a potentially advantageous delivery system for brain tumour chemotherapy[83]. It has also demonstrated that 85 nm liposomes can be sterically stabilized with a 2000 dalton PEG that contains lipid at one end and maleimide at the end distal to the liposome. Monoclonal antibodies (Mabs) can be coupled with a sulfide linkage to the maleimide moiety of these stealth liposomes to form immunoliposomes. The OX26 Mab has been linked with stealth liposome to cross the BBB through transferrin receptor and used for delivery of neuropharmaceuticals[84].

Initially conventional liposomes have been rejected because of low brain delivery or some minor side effects. Above mention delivery system may be significant to brain drug delivery because it permits brain targeting of a liposomal encapsulated drug and may consequently offer a significant reduction in side effects.

Nanoparticles:

An alternative approach is the employment of nanoparticles. Nanoparticles are solid colloid particles ranging 1 to 1000 nm in size[85]. They consist of macromolecular materials in which the active principle is dissolved, entrapped or encapsulated or to which the active principle is adsorbed or attached. The mechanism of this transport has not yet been fully elucidated. In principle, six different possibilities exist that could enable enhancement of the drug transport across the BBB by means of nanoparticles.

1. Nanoparticles are preferentially adsorbed on the wall of the brain blood vessel without transport of particles across the endothelium. Simple adhesion of the nanoparticle to the brain blood vessel walls leads to significant drug transport to the brain. The adhesion of the nanoparticle might be observed in higher brain radioactivity level with polysorbate 80 coated ^{14}C-labelled nanoparticles[85].

2. The fluidization of the endothelium by the surface activity of the surfactant polysorbate 80 enhances the drug transport across the brain. But this possibility become less prominent when other polysorbates like 83 and 85 and other surfactants are used. It may be argued that the polysorbate would interact much more strongly with the nanoparticle than the other surfactant[86].

3. Another possibility of the enhanced transport of the drug across the BBB is opening of the tight junction between the endothelium cells lining the blood brain vessels. The junction may be opened for instance by hyperosmotic pressure, results enhanced drug transport into the brain[87-89].

4. At present the most likely mechanism for the brain transport of drugs seems to be endocytotic uptake by the endothelial cells lining of the brain blood vessels. These cells belong to classical reticuloendothelium system and are responsible for endocytosis of particulate matter under certain circumstances[90]. After endocytosis, delivery of the drug to the other brain cells may occur by desorption of the drug from the nanoparticle with or without degradation of the nanoparticle. The later is possible since the polymer employed is very rapidly biodegradable i.e. poly (butylcynoacrylate)[91,92]. Following its release by desorption or biodegradation, the drug would enter the residual brain by diffusion. Alternatively, transport into these parts of the brain could occur by transcytosis of the nanoparticle with drug across the endothelium cells[93].

5. Another approach for drug delivery to brain is transcytosis across the brain endothelial cells. After the uptake of the nanoparticle by the endothelium cells, the nanoparticles and adsorbed drug may be delivered to the other brain cells by transcytosis of the nanoparticles. LDL particles may be transported across the BBB by receptor mediated transcytosis in the endothelium is different from the LDL receptor classic pathway[93].

6. The inactivation of P-glycoprotein efflux pump would enhance the brain transport of nanoparticle. P-glycoprotein, which is responsible for multidrug resistance of tumour cells is also present in the brain endothelium cells[94] and represents a major obstacle to cancer therapy[95-97]. Surfactants including polysorbate 80 were shown to inhibit this efflux system and reverse the multidrug resistance[95-98].

Drugs that have successfully been transported across this barrier by the nanoparticles include dalargin[99,100], loperamide[101] and tubocurarine[102]. The coating of nanoparticles with surfactants like polysorbate 80 and 20, poloxamers 188, 338, 407 and 184 polyoxyethylene, 23-laural ether and poloxamine 908 offers the possibility to increase the brain concentration after intravenous injection[103,104].

Nano-conjugates:

These are low molecular weight conjugates of a small drug or toxin and a targeting ligand coupled through a cleavable linker group, which is consisted of three functional domain, the targeting group: a linker; and an active agent/drug. Typically nano conjugates have a molecular weight similar to that of standard cytotoxic drugs (<3 kDa). Drug transport and distribution in the interstitium are based on convection and diffusion. The convective transport of molecules is independent of their molecular

weight, carrying molecules in the flow of blood or interstitial fluid. Solid tumours frequently have an increased interstitial pressure that severely limits convection-based transport of drugs[105-107]. The efficiency of drug transport through diffusion on the other hand is inversely proportional to the molecular weight of the drug/carrier. Thus, small molecules and nano-conjugates have potential advantages in situations where drug transport is diffusion limited[108,109].

Chemical delivery systems:

Brain targeted chemical delivery systems (CSDs) represent a rational drug design approach that exploits sequential metabolism not only to deliver but also to target drugs to their site of action[4,110]. By localizing drugs at their desired site of action one can reduce toxicity and increase treatment efficiency. The CDS concept evolved from the prodrug concept on the early 1980s, but was differentiated by the introduction of target moieties and the use of multistep activation[111]. The cunning aspect of these brain targeted systems is that in addition to providing access by increasing the lipophilicity, they exploit the specific bi-directional properties of the BBB to 'lock in' active drug precursors in the brain on arrival, preventing exit back across the BBB. CDSs are inactive chemical derivatives of a drug being obtained by one or more chemical modifications. The newly attached moieties are monomolecular units that provide a site specific or site enhanced delivery of the drug through multistep enzymatic and /or chemical transformations. Brain targeting CDSs exploit the fact that if a lipophilic compound enters the brain and is then converted into lipophobic molecule it will no longer be able to exit and it will be locked in. In principle, many targeting moieties are possible for a general system of this kind but the one which is based on the 1,4–dihydrotrigonelline–trigonelline system. The conversion takes place easily because it is closely related to that of the ubiquitous NADPH—NADP coenzyme system. It provides a non-toxic targetor system because oxidation occurs with direct hydride transfer and without generating highly active or reactive radical intermediates. Although, the charged intermediate T*+D consisting of the quaternary targetor (T*) and drug (D) complex is locked behind the BBB into the brain, it is easily eliminated from the body because it acquires the positive charge, which enhances water solubility. After a relatively short time the delivered drug D is present only in the brain providing brain specific availability of the active drug[112].

CONCLUSIONS

A great deal of previous work on drug delivery to BBB has been directed to the strategy of increasing lipophilicity of drugs, which is based on the assumption that transport of drugs across the BBB is diffusion-limited. However, recent advances in studies on the BBB transport of drugs, have led to a great change in the old concept of BBB. Three strategies have developed to compensate the problem of BBB. The intravenous infusion by invasive neurosurgical procedure is an alternate approach to overcome the disease. However, the risk of infection, catheter clotting, neurosurgical cost, diffusion limitations beyond the ventricular surface to the parenchyma of the brain where the therapeutic agents exert their effect and rapid clearance by the cerebrospinal fluid, emphasize the considerable need to develop novel form of brain drug delivery systems. The disruption of BBB by an osmotically active agent or by a vasoactive agent or by any other means causes a transistory opening of the BBB that may lead to the influx of serum protein into the brain interstitial fluid. The second strategy seeks to improve the drug uptake by the brain by modifying the physiochemical properties of the therapeutic drug. Lipophilicity, efflux pump, molecular charge and molecular weight determine the extent to which a drug can cross the BBB. Delivery of these molecules i.e. high molecular weight or hydrophilic drug can be circumvented by the use of carrier such as cationized proteins, monoclonal antibodies or peptides. In vivo use of peptides vectors such as those referred to in this review should be given serious consideration there small size, ease of drug attachment and non-invasive transport into the central nervous system make them the vectors of choice for the delivery of drug to the brain.

ACKNOWLEDGEMENTS

One of the authors is thankful to Council of Scientific and Industrial Research, New Delhi, India for providing financial assistance to carry out project on brain targeting of drugs.

REFERENCES

1. Goldstein, G.W. and Betz, A.L., **Sci. Amer.**, 1986, 5, 74.
2. Begley, D.G., **J. Pharm. Pharmcol.**, 1996, 48, 136.
3. Buchwald, P. and Bodor, N., **Cur. Med. Chem.**, 1998, 5, 353.
4. Bodorn, N. and Buchwald, P., **Advr. Drug Delivery Rev.**, 1999, 36, 229.
5. Neuwelt, E.A., Goldman, S.A., Delborg, J.C., Ramsey, F., Goldstein, R.A., Braziel, R. and Dana, B., **J. Clin. Oncol.**, 1991, 9, 1580.
6. Salahuddin, T.S., Kalimo, H. and Olsson, Y., **Acta. Neuropathol.**, 1988, 76, 1.
7. Salahuddin, T.S., Kalimo, H., Johansson, B.B. and Olsson, Y.,

Acta. Neuropathol., 1988, 77, 5.
8. Richmon, J.D., Fukuda, K., Sharp, F.R. and Noble, L.J., **Neurosci. Lett.**, 1995, 202, 1.
9. Neuwelt, E.A, Minna, J., Frenkel, E. and Barnett, P.A., **Amer. J. Physiol.**, 1986, 250, R875.
10. Neuwelt, E.A., Specht, H.D., Dahlberg, S.A., and Barnett, P.A., **J. Neurosurg.**, 1987, 20, 885.
11. Neuwelt, E.A., Barnett, P.A., Ramsey, F., and McCormick, C.L., **J. Nucl. Med.**, 1994, 35, 1831.
12. Blake, K.L., Advn. **Drug Delivery Rev.**, 1995, 15, 37.
13. Inamura, T. and Blake, K.L., **J. Cerebr. Blood Flow Metabol.**, 1994, 14, 862.
14. Anatomy, D. and Dobson, **P., Life Sci.**, 1984, 35, 2135.
15. Brem, H., **J. Neurosurg.**, 1991, 74, 441.
16. Brem, H., **Lancet**, 1995, 345, 1008.
17. Tamargo, R.G. and Myseros, J.S., **Cancer Res.**, 1993, 53, 329.
18. Sipos, E. P. and Tylor, B., **Cancer Chemother. Pharmacol.**, 1997, 39, 383.
19. Judy, K.D. and Olivi, A., **J. Neurosurg.**, 1995, 82, 481.
20. Walter, K.L. and Cahen, M.A., **Cancer Res.**, 1994, 54, 2207.
21. Weingart, J.D. and Thomson, R.C., **Int. J. Cancer**, 1995, 62, 605.
22. Weingart, J.D. and Sipos, E.P., **J. Neurosurg.**, 1995, 82, 635.
23. Olivi, A. and Utsuki, M.G., **Cancer Chemother. Pharmacol.**, 1996, 39, 90.
24. Ewend, M.G. and Willioms, J.A., **Cancer Res.**, 1996, 56, 5217.
25. Wallner, K.E. and Galicich, J.H., **Int. J. Radiat. Oncol. Biol. Phys.**, 1989, 16, 1405.
26. Gise, A. and Westphal, M., **J. Neurosurger.**, 1996, 39, 235.
27. Hochwerg, F.H. and Pruitt, A., **Neurology**, 1980, 30, 907.
28. Fung, L.K. and Shin, M., **Pharm. Res.**, 1996, 13, 671.
29. Strasser, J.F. and Fung, L.K., **J. Pharmacol. Exp. Ther.**, 1995, 275, 1647.
30. Grossman, S.A. and Reinhard, C., **J. Neurosurg.**, 1992, 76, 640.
31. Domb, A. and Rock, M., **Biomaterials**, 1994, 15, 681.
32. Buahin, K.G. and Judym, K.D., **Adv. Technol.**, 1992, 3, 311.
33. Fung, L.K. and Ewend, M., **Cancer Res.**, 1998, 58, 672.
34. Begley, D.J., **J. Pharm. Pharmcol.**, 1988, 37, 2973.
35. Tsuji, A. and Tamai, H., **Advan. Drug. Delivery Rev.**, 1999, 36, 277.
36. Wade, L.A. and Katzman, R., **J. Neurochem.**, 1975, 25, 837.
37. Horen, P.J., Matta, A., Bilsky, E.J. and Davis, T.P., **J. Pharmacol. Exp. Ther.**, 1993, 265, 1446.
38. Silbert, B.S., Lipkowski, A.Q., Cepda, M.S. and Carr, D.B., **Agents Actions**, 1991, 33, 382.
39. Abbriscato, T.J., Thomas, S.A., Hurdy, V.J. and Davis, T.P., **J. Neurochem.**, 1997, 69, 1236.
40. Kenn, A.W., Terrence, J.G., Jason, D.H., Richered, D.E. and Davis, **T.P., Peptide**, 2001, 22, 2329.
41. Frists, A., Wolf, D. and Brett, G.M., **J. Pharmacol. Exp. Ther.**, 2000, 52, 207.
42. Bickel, U. and Pardridge, W.M., **Proc. Nat. Aca. Sci.**, 1993, 90, 2618.
43. Shin, S.U. and Pardridge, W.M., **J. Immunol.**, 1997, 158, 4797.
44. Penichet, M.L. and Pardridge, W.M., **J. Immunol.**, 1999,.163, 4421.
45. Vinogradov, S. and Betrakova, E., **Bioconjug. Chem.**, 1999, 10, 851.
46. Kang, Y.S. and Pardridge, W.M., **Pharm. Res.**, 1994, 11, 1257.
47. Ress, A.R. and Webster, D.M., **Biotechnol.**, 1994, 12, 199.
48. Chester, K.A. and Howkins, R.E., **Biotechnol.**, 1995, 13, 294.
49. Melton, R. and Sherwood, R., **J. Nat. Cancer Inst.**, 1996, 88, 153.
50. Niculscu, D.I. and Springer, C., **Cur. Med. Chem.**, 1996, 2, 687.
51. Siemer, N. and Kerr, D., **Bioconju. Chem.**, 1997, 8, 510.
52. Pietersz, G. and Rowland, A., **Adv. Immunol.**, 1994, 56, 301.
53. Svensson, I.I. and Crudhula, V., **Cancer Res.**, 1995, 55, 2357.
54. Kerr, D. and Schreiber, G., **Cancer Res.**, 1995, 55, 3558.
55. Pardridge, W.M., **Endocrine Rev.**, 1986, 7, 314.
56. Pardridge, W.M., In; Simionescu, N. and Simionescu, M., Eds. Endothelial cell Dysfunctions, Plenum Press, New York, 1992, 347.
57. Fishman, J.B., Rubin, J.B., Handrahen, J.V., Connor, J.R. and Fine, R.E., **J. Neurosci. Res.**, 1987, 48, 299.
58. Fenart, L., Dehouck, M.P., Dehouck, B., Torpier, G. and Cocchelli, R., In; Pardridge W. M., Eds., Introduction to the BBB, Cambridge University Press, Cambridge, 1998, 221.
59. Banks, W.A., Kastin, A.J., Huang, W., Jaspan, J.B. and Maness, L., **Peptides**, 1996, 17, 305.
60. Pardridge, W.M., In; Peptide drug delivery to brain, Raven Press, New York, 1991, 198.
61. Golden, P.L., Macccagnan, T.J. and Pardridge, W.M., **J. Clin. Invest.**, 1997, 99, 14.
62. Skarlatos, S., **Brain Res.**, 1995, 683, 164.
63. Bickel, U., In; The BBB and drug delivery to the CNS, Marcel Dekker, Ins., 2001, 171.
64. Pardridge, W.M., **Pharm. Sci. Today**, 1999, 2, 49.
65. Pardridge, W.M., **Pharmaceut. Res.**, 1994, 11, 738.
66. Kang, Y. S., Boado R. J., and Pardridge, W.M., **Drug Metab. Dispos.**, 1995, 23, 55.
67. Bangham, A.D., **J. Mol. Biol.**, 1965, 13, 238.
68. Gabizon, A.A., and Papahajopoulos, **D. Proc. Nat. Aca. Sci.**, 1988,85,6949.
69. Greoriadis, G., **FEBS Lett.**, 1973, 36, 292.
70. Greoriadis, G., Leathwood, P.D. and Ryhem, B.E., **FEBS Lett.**, 1971, 14, 95.
71. Greoriadis, G. and Allison, A.C., **FEBS Lett.**, 1974, 45, 71.
72. Kimelberg, K.G., **Biochem. Biophy. Acta**, 1976, 448, 531.
73. Papahajopoulos, D. and Vali, W.J., **Ann. N. Y. Acad. Sci.**, 1978, 308, 259.
74. Greoriadis, G. and Aengl, N., **J. Med.**, 1976, 295, 704.
75. Bruni, A., Leon, A. and Boardato, E., In; function and metabolism of Phospholipids in the central and peripheral nervous system, Plenum Press, New York, 1976, 271.
76. Daoud, S. and Hume, L., **Adv. Drug Delivery Rev.**, 1989, 3, 405.
77. Sells, R.A., **Lancet**, 1989, 11, 624.

78. Allen T.M., **Trends Pharmacol. Sci.**, 1994, 15, 215.
79. Gabizen, A.A., **Cancer Res.**, 1992, 52, 981.
80. Gabizon, A.A., **Hematol. Clin. North. Amer.**, 1994, 8, 431.
81. Vagee, J., Mayheoo, E.M. and Lasic, D.D., **Int. J. Cancer**, 1992, 51, 942.
82. Williams, S.S. and Alocco, T.R., **Cancer Res.**, 1993, 53, 3964.
83. Sigal, T. and Aviva, M.D., **J. Neurosurg.**, 1995, 83, 1029.
84. Huwyler, J., Wu, D. and Padridge, W.M., **Proc. Nat. Aca. Sci.**, 1996, 93, 14164.
85. Kreuter, J. In; Swarbic, J., Boylan, J.C. Eds. Encyclopaedia of Pharmaceutical Technology, Vol. 10, Marcel Dekker, New York, 1994, 165.
86. Kreuter, J., Petrov, V.B., Kharkevich, D.A. and Alyautdin, R.N., **J. Control. Release**, 1997, 49, 81.
87. Neuwelt, E.A. and Barnett, P.A., In; Neuwelt, E. A., Eds., Implication of BBB and its manipulation, Vol. 2, Plenum Press, New York, 1989, 195.
88. Neuwelt, E.A., Barnett, P.A., Pegal, M., Glassberg, M. and Frenkel, E.P., **Cancer Res.**, 1981, 41, 4466.
89. Neuwelt, E.A., Glassberg, M. and Frenkel, E.P., **Ann. Neurol.**, 1983, 14, 316.
90. Saba, T.M., **Intern. Med.**, 1970, 126, 1031.
91. Grislain, L., Couvereur, P., Lenaerts, V., Roland, M. and Deprez, D., **Int. J. Pharm.**, 1983, 15, 335.
92. Couvereur, P., Grislain, L., Lenaerts, V., Brasseur, F., Guiot, P. and Biernacki, A., In: Guiot, P. Couvreur, P., Eds. Polymeric Nanoparticles and Microspheres, Boca Raton FL CRS Press, 1986, 27.
93. Dehouck, B., Fenart, C.D., Ehouck, M.P., Pierce, A., Torpier, G. and Cecchelli, R., **J. Cell Biol.**, 1997, 138, 887.
94. Cordon, C., O'Brien, J.P., Casals, D., Biedler, J.L., Melamed, M.R. and Bertino, J.R., **Proc. Natl. Acad. Sci.**, 1989, 86, 695.
95. Woodcock, D.M., Linsenmeyer, M.E., Jefferson, S. and Crowther, P.J., **J. Cancer Res.**, 1990, 50, 4199.
96. Woodcock, D.M., Linsenmeyer, M.E., Chojnowski, G.M., Kriegler, A.B., Nink, V., Webster, L.K. and Sawyer, W.H., **Brit. J. Cancer**, 1992, 66, 62.
97. Zordon-Nudo, T., Ling, V., Liv, Z. and Georges, E., **Cancer Res.**, 1993, 53, 5994.
98. Nerurkar, M.M., Burto, P.S. and Borchardt, R.T., **Pharm.Res.**, 1996, 13, 528.
99. Kreuter, J., Alyautdin, R.N., Kharkevich, D.A., and Ivanov, A.A., **Brain Res.**, 1995, 674, 171.
100. Alyautdin, R.N., Gothier, D., Petrov, V. E., Kharkevich, D.A. and Kreuter, J., **Eur. J. Pharm. Biopharm.**, 1995, 41, 44.
101. Alyautdin, R.N., Petrov, V. E., Langer, A. K., Berthold, A. and Kharkevich, D.A., **Pharm. Res.**, 1997, 14, 325.
102. Alyautdin, R.N., Tezikov, E.B., Ramge, P., Kharkevich, D.A., Begley, D.J. and Kreuter, J., **J. Microencapsulation**, 1998, 15, 67.
103. Troster, S.D., Muller, U. and Kreuter, J., **Int. J. Pharm.**, 1990, 61, 85.
104. Troster, S.D. and Kreuter, J., **J. Microencapsulation**, 1992, 9, 19.
105. Juweid, M., Neumann, R., Paik, C. and PeRez-Bacete, M.J., **Cancer Res.**, 1992, 52, 5144.
106. Jain, R.K. and Baxter, L.T., **Cancer Res.**, 1988, 48, 7022.
107. Shokley, T.R., Lin, K., Nagy, J.A., Tompkins, R.G., Yarmucsh M.L. and Dverac, H.F., **Cancer Res.**, 1992, 52, 367.
108. Jain, R.K., **J. Control. Release**, 1998, 53, 49.
109. Aloj, L., Jogoda, E., Lang, L., Caraco, C., Neumann, R.D., Sung, C. and Eckrlmen, W.C., **J. Nucl. Med.**, 1999, 40, 1547.
110. Lindgren, M., **Trends Pharmacol. Sci.**, 2000, 21, 99.
111. Bordor, N. and Brewster, M.E., In; Juliano, R.L., Eds., Targetted Drug Delivery. Sprilnger Berlin, 1991, 231.
112. Nicholas, B. and Buchwald, P., **Drug Deliv. Today**, 2002, 7, 766.