DRUG TARGETING TO BRAIN : A REVIEW

Daljeet Sharma¹, Narendra Nager¹, Ravindra Pal Singh¹, Ashish Kumar Sharma¹

¹Department Of Pharmacy
Suresh Gyan Vihar University, Jaipur
Rajasthan, India
Email: daljeetsharma@ymail.com

ABSTRACT

The challenge in drug targeting is not only the targeting of drug to a specific site but also retaining it for the desired duration to elicit pharmacological action. For a nanosystem administered intravenously, the first and foremost barrier is that of the vascular endothelium and the basement membrane. Also, plasma proteins have the ability to affect the biodistribution of drug carrier systems introduced in the blood stream. The in vivo biodistribution and opsonization of nanosystems in blood circulation is governed by their size and surface characteristics. For the nanosystem to remain in blood circulation for a long time, the major problem is to avoid its opsonization and subsequent uptake by the phagocytic cells. The tight endothelial cells in brain constitute the blood brain barrier (BBB), which restricts the entry of most drugs and delivery systems. However vascular endothelium is not uniform throughout. Another barrier is that of the extracellular matrix, which should be crossed to access the target cells in a tissue. If the whole tissue constitutes a target then the uniform distribution of drug throughout the tissue is another problem.

Key Words: BBB, Biodistribution, nanoparticals, brain, nanosystem.

INTRODUCTION

The blood-brain barrier (BBB) is a membranic structure in the central nervous system (CNS) that restricts the passage of various chemical substances and microscopic objects (e.g. bacteria) between the bloodstream and the neural tissue itself. This "barrier" results from the selectivity of the tight junctions between endothelial cells in CNS vessels that restricts the passage of solutes. At the interface between blood and brain, endothelial cells and associated astrocytes are stitched together by structures called tight junctions. The tight junction is composed of smaller subunits,
frequently dimers, that are transmembrane proteins such as occludin, claudins, junctional adhesion molecule (JAM), ESAM and others. Each of these transmembrane proteins is anchored into the endothelial cells by another protein complex. The blood-brain barrier is composed of high density cells restricting passage of substances from the bloodstream much more than endothelial cells in capillaries elsewhere in the body. Astrocyte cell projections called astrocytic feet (also known as "glia limitans") surround the endothelial cells of the BBB, providing biochemical support to those cells.

The BBB is distinct from the similar blood-cerebrospinal fluid barrier Ehrlich was a bacteriologist who was studying staining, used for many studies to make fine structures visible. When injected, some of these dyes (notably the aniline dyes that were then popular) would stain all of the organs of an animal except the brain. At the time, Ehrlich attributed this to the brain simply not picking up as much of the dye.

( Diagram of a cerebral capillary enclosed in astrocyte end-feet. Characteristics of the blood-brain barrier are indicated: (1) tight junctions that seal the pathway between the capillary (endothelial) cells; (2) the lipid nature of the cell membranes of the capillary wall which makes it a barrier to water-soluble molecules; (3), (4), and (5) represent some of the carriers and ion channels; (6) the 'enzymatic barrier' that removes molecules from the blood; (7) the efflux pumps which extrude fat-soluble molecules that have crossed into the cells.)

BLOOD BRAIN BARRIER

Physiology

The blood-brain barrier acts very effectively to protect the brain from many common bacterial infections. Thus, infections of the brain are very rare. However, since antibodies are too large to cross the blood-brain barrier, infections of the brain which do occur are often very serious and difficult to treat. Viruses easily bypass the blood-brain barrier, however, attaching themselves to circulating immune cells.

Drugs Targeting The Brain

Overcoming the difficulty of delivering therapeutic agents to specific regions of the brain presents a major challenge to treatment of most brain disorders. In its neuroprotective role, the blood-brain barrier functions to hinder the delivery of many potentially important diagnostic and therapeutic agents to the brain. Therapeutic molecules and genes that might otherwise be effective in diagnosis and therapy do not cross the BBB in adequate amounts.

Mechanisms for drug targeting in the brain involve going either "through" or "behind" the BBB. Modalities for drug delivery through the BBB entail its disruption by osmotic means, biochemically by the use of vasoactive substances such as bradykinin, or even by localized exposure to high intensity focused ultrasound (HIFU).
Other strategies to go through the BBB may entail the use of endogenous transport systems, including carrier-mediated transporters such as glucose and amino acid carriers; receptor-mediated transcytosis for insulin or transferrin; and blocking of active efflux transporters such as p-glycoprotein. Strategies for drug delivery behind the BBB include intracerebral implantation and convection-enhanced distribution.

NANOPARTICLES:

Nanotechnology may also help in the transfer of drugs across the BBB. Recently, researchers have been trying to build nanoparticles loaded with liposomes to gain access through the BBB. More research is needed to determine which strategies will be most effective and how they can be improved for patients with brain tumors. The potential for using BBB opening to target specific agents to brain tumors has just begun to be explored. Delivering drugs across the blood brain barrier is one of the most promising applications of nanotechnology in clinical neuroscience. Nanoparticles could potentially carry out multiple tasks in a predefined sequence, which is very important in the delivery of drugs across the blood brain barrier. A significant amount of research in this area has been spent exploring methods of nanoparticle mediated delivery of antineoplastic drugs to tumors in the central nervous system. For example, radiolabeled polyethylene glycol coated hexadecylcyanoacrylate nanoparticles targeted and accumulated in a rat gliosarcoma. However, this method is not yet ready for clinical trials due to the accumulation of the nanoparticles in surrounding healthy tissue. Another, recent effort with the nanoparticle mediated delivery of doxorubicin to a rat glioblastoma has shown significant remission as well as low toxicity. Not only is this result very encouraging, but it could lead to clinical trials.

Not only are nanoparticles being utilized for drug delivery to central nervous system ailments, but they are also being investigated as possible agents in imaging. The use of solid lipid nanoparticles consisting of microemulsions of solidified oil nanodrops loaded with iron oxide could increase in MRI imaging because of the ability of these nanoparticles to effectively cross the blood brain barrier.

BLOOD BRAIN BARRIER DRUG TARGET:

The development of new drugs for the brain has not kept pace with progress in the molecular neurosciences, because the majority of new drugs discovered do not cross the blood-brain barrier (BBB). Although approximately 100% of large-molecule drugs do not cross the BBB, the problem is nearly as severe for small-molecule drugs—greater than 98% of small-molecule drugs do not cross the BBB.

Rate-Limiting Role of The BBB In Brain Drug Development

The rate-limiting role of the BBB is illustrated with histamine, a small molecule of only 111 Da. Histamine, however, does not cross the BBB, and the inability of histamine to penetrate the brain. Histamine has too many hydrogen-bond-forming functional groups, and BBB penetration is inversely related to the number of hydrogen...
bonds that a drug forms with solvent water. Molecules that do cross the BBB typically are lipid soluble and have a $M_r$ threshold of 400-500 Da.

**The Limitations of Small–Molecule Drugs**

Drug companies today do not have in-house BBB drug targeting programs because it is widely believed that most disorders of the brain respond to small molecules and most small molecules cross the BBB. However, only a few brain diseases consistently respond to lipid-soluble small molecules. This fact is illustrated by several reviews of current CNS drugs. In one study of the comprehensive medicinal chemistry (CMC) database, over 7,000 drugs were analyzed and only 5% of these drugs affected the CNS, and these CNS-active drugs treated only depression, schizophrenia, and insomnia. The average $M_r$ of the CNS active drug was 357-Da. In another study, only 12% of drugs were active in the CNS and only 1% of the total number of drugs were active in the CNS for diseases other than affective disorders.

(Structure of the Blood–Brain Barrier (BBB).

**Craniotomy-Based Brain Drug Delivery**

There are examples of CNS drug development programs that go forward even though it is known that the drug does not cross the BBB and that no BBB drug delivery strategy is available. In this setting, the strategy for dealing with the BBB problem is to administer the drug after drilling a hole in the head, a process called craniotomy. With this approach, the small- or large-molecule drug may be administered either by intracerebroventricular (ICV) or intracerebral (IC) injection. With IC administration, the drug stays at the depot site at the tip of the injection needle or at the margins of the polymeric implant. With ICV administration, the drug only distributes to the ependymal surface of the ipsilateral ventricle and does not significantly penetrate into brain parenchyma. Thus, the treatment volume with either ICV or IC administration is less than 1% of the brain volume, and there are few, if any, brain diseases that are treatable with such limited penetration of drug into the brain.

(Transcranial and transvascular drug delivery to the brain.)

**The Vascular Route To The Brain**

In contrast to the inefficiency of craniotomy-based drug delivery to the brain, a transvascular route of drug administration, following intravenous or systemic injection, can treat virtually 100% of the neurons in the brain. Because every neuron is perfused by its own blood vessel, the drug is delivered to the "doorstep" of every neuron in the brain following initial transport across the vascular barrier. In the human brain, there are approximately 100 billion capillaries totaling 400 miles in length. The combined surface area of brain capillary endothelium is approximately 20 m$^2$ in the
human brain. The delivery of drugs (or genes) to the brain by the transvascular route is so efficient that the drug or gene could be delivered to all parts of the brain once the vascular barrier is traversed. However, in the absence of a BBB drug-targeting system, the transvascular route to the brain is virtually impenetrable by the majority of drug candidates. If the large numbers of patients worldwide that are afflicted with serious disorders of the brain and spinal cord are to be treated, then the present trend of persistent under-development of BBB transport biology must be reversed.

Outline Of A Blood Brain Barrier Drug Targeting Program

There are both chemistry-based and biology-based approaches for developing BBB drug-targeting strategies. The chemistry-based strategies are the conventional approaches that rely on lipid-mediated drug transport across the BBB. The limitations of lipid-mediated BBB drug transport are discussed below. The biology-based approaches require prior knowledge of the endogenous transport systems within the brain capillary endothelium, which forms the BBB in vivo. The biology-based strategies for brain drug delivery are founded on the principle that there are numerous endogenous transport systems within the BBB, and that these transporters are conduits to the brain. The endogenous BBB transport systems may be broadly classified as carrier-mediated transport (CMT), active efflux transport (AET), and receptor-mediated transport (RMT). These BBB transport systems are situated on the luminal and abluminal membranes of the brain capillary endothelium.

Drug delivery to the brain through the many endogenous transport systems within the BBB requires reformulation of the drug so that the drug can access the BBB transport system and enter the brain.

Chemistry Based Approach: BBB Lipid-Mediated Transport

There are two ways that a drug can be lipidated. First, the polar functional groups on the water-soluble drug can be masked by conjugating them with lipid-soluble moieties. Second, the water-soluble drug can be conjugated to a lipid-soluble drug carrier. Either reformulation of the drug leads to the production of a prodrug, which is lipid soluble and can cross the BBB. Ideally, the prodrug is metabolized within the brain and converted to the parent drug. Apart from the diacetylation of morphine to create heroin, there have been few examples wherein the prodrug approach has been used to successfully solve the BBB drug-delivery problem in clinical practice. Two limitations of the prodrug approach are the adverse pharmacokinetics and the increased molecular weight of the drug that follow from lipidation.
The Pharmacokinetic Rule

The percent of injected dose (ID) of a drug that is delivered per gram brain (%ID/g) is directly proportional to both the BBB permeability–surface area (PS) product and the area under the plasma concentration curve (AUC):

\[
% \text{ID}/g = PS \times AUC
\]

When a drug is lipidated, the BBB PS product is increased. However, the penetration of the lipidated drug is also increased in all organs of the body, which alters the plasma clearance of the drug. Following lipidation, the blood half-time of a drug may decrease from several hours to only a few minutes. Thus, there is a reduction in the plasma AUC in parallel with the increase in membrane permeation caused by lipidation. The increased PS product and the decreased plasma AUC have offsetting effects leading to nominal increases in the % ID/g of brain, which is not increased in proportion to the increase in BBB PS product or lipid solubility.

MOLECULAR WEIGHT THRESHOLDS

The conversion of a water-soluble drug into a lipid-soluble prodrug leads to an increase in the molar mass of the drug. This increase in M, can be substantial depending on the strategy used to lipidate the drug. The molecular weight of virtually all CNS-directed drugs in present-day clinical practice are under 400–500 Da. Lipid-soluble drugs with masses above the 400–500 Da threshold, with some exceptions, do not cross the BBB in pharmacologically significant amounts.

The biophysical basis of the mass-specific threshold of BBB drug transport is explicable within the context of a pore model of lipid-mediated transport across biological membranes. The membrane phospholipid bilayer is not inert but is mobile in living cells. This mobility causes kinks in the long chain fatty acyl groups that create transient pores within the membrane to enable "molecular hitchhiking" of the lipid-soluble small-molecule drugs across biological membranes. This model would not be applicable for drug diffusion through solvents, which reinforces the idea that drug diffusion across biological membranes is not effectively modeled by drug diffusion through solvents, particularly when the molecular mass of the drug exceeds 400-Da. The permeation of a drug through a biological membrane decreases exponentially as the molecular size of the drug increases. For BBB transport, the upper limit in molecular area appears to be about 80 Å², which corresponds to a M, of less than 300–400 Da. If the size of the drug is doubled from 50 Å² (M, about 250-Da) to 100 Å² (M, about 400-Da), the BBB permeation decreases by 100-fold.

Biology-Based Approach: BBB Carrier Mediated Transport

The conversion of dopamine, a water-soluble catecholamine that does not cross the BBB, into the corresponding α-amino acid, L-DOPA, enables dopamine delivery to the brain, which has been the mainstay of treatment of PD for nearly 40 years. The use of L-DOPA to deliver dopamine to the brain is a BBB drug-delivery strategy that utilizes the type 1 large neutral amino acid transporter (LAT1)—one of the CMT systems within the BBB. Upon crossing the BBB through LAT1, L-DOPA is converted back to dopamine within the brain by aromatic amino acid decarboxylase (AAAD).

Other drugs that cross the BBB via LAT1 include melphalan for brain cancer, α-methyl-DOPA for treatment of high blood pressure, and gabapentin for epilepsy. Apart from LAT1, there are other BBB CMT systems that could be accessed to solve BBB drug-delivery problems.
including the GLUT1 glucose transporter, the MCT1 lactate transporter, the CAT1 cationic amino acid transporter, and the CNT2 adenosine transporter, among others. If the BBB CMT systems are to be exploited to overcome the BBB drug-delivery problem, the drug must be reformulated such that the drug assumes a molecular structure mimicking that of the endogenous ligand.

This principle is illustrated by gabapentin, which is 1-(aminoethyl) cyclohexaneacetic acid. Gabapentin is a γ-amino acid, not an α-amino acid. However, this drug’s structure does mimic that of an α-amino acid and is recognized by the BBB LAT1 large neutral amino acid transporter. In the absence of LAT1-mediated transport across the BBB, gabapentin would be too water soluble to cross (via lipid mediation) the BBB in pharmacologically significant amounts.

**Enzymatic BBB**

The different components of the "enzymatic BBB" must be considered in addition to the endogenous BBB transport systems when designing brain drug delivery strategies. The enzymatic systems that degrade molecules crossing the endothelial membrane may be expressed on the endothelial plasma membrane, the pericyte plasma membrane, or the astrocyte foot process.

The brain capillary endothelial cell and the brain capillary pericyte, which sits on the brain-side of the endothelium, share a common microvascular basement membrane. Nearly 100% of the surface area of the capillary basement membrane is covered by end-feet of processes originating from brain astrocytes, and these astrocytic end-feet are separated from the capillary endothelium by a distance of only 20 nm. In fact, the endothelium, the pericyte, and the astrocyte foot process work in concert to tightly regulate the flux of molecules between blood and brain across the microvascular barrier.

**Molecular Biology of BBB Carrier-Mediated Transporters**

Some of the BBB CMTs have been cloned, and from their full-length cDNAs, RNA is transcribed and prepared that can be injected into frog oocytes for the expression of BBB transporters. This methodology enables the measurement of the transport kinetics of these transporter proteins. The complementary RNA (cRNA) from BBB CNT2 is particularly active in frog oocytes and enabled the detailed kinetic analysis of the transport of dideoxyinosine (DDI) via the CNT2 transporter.

This molecular biological approach to BBB CMT systems is to be preferred over in vitro BBB models. Although brain capillary endothelial cells may be grown in tissue culture to form an "in vitro BBB", the gene expression of many of the BBB CMT systems is severely downregulated in tissue culture. Indeed, the transport of L-DOPA across the BBB by the CMT (e.g., LAT1) system would probably not be detected in an in vitro BBB screen, owing to decreased gene expression of the BBB LAT1 in brain endothelium grown in tissue culture.

**Biology-Based Approach: BBB Active Efflux Transport**

P-glycoprotein (Pgp) is the prototypic AET system found at the BBB. However, there are many other AETs other than Pgp that function at the BBB to cause the selective export of metabolites from brain back to blood. Although Pgp is principally expressed at the capillary endothelium in rodent brains, this transporter is also expressed at both the capillary endothelium and at astrocyte processes in primate and human brains. Within
the brain capillary endothelium, it is assumed that Pgp is selectively localized at the luminal membrane, although the definitive immunogold electron-microscopic studies for this transporter have yet to be performed for brain.

The GLUT1 glucose transporter is expressed at both the luminal and abluminal endothelial membranes in rat brain, and this transporter comigrates with Pgp in fractionated plasma membranes from rat brain endothelia.

Polarity of BBB Active Efflux Transporters

Active efflux transport at the BBB is likely the result of the concerted action of energy-dependent and energy-independent transport systems selectively localized to the luminal and abluminal endothelial membranes, similar to the polarity of glucose transporters at the apical and basolateral membranes of renal tubular epithelium. Energy-independent exchangers may be expressed at the abluminal membrane and work in conjunction with ATP-dependent transporters, such as Pgp, at the luminal membrane.

Alternatively, sodium-dependent co-transporters may be expressed at the abluminal membrane and work in concert with energy-independent exchangers at the luminal endothelial membrane. Candidates for energy-dependent active transporters at the BBB include Pgp or certain multi-drug resistance proteins (MRPs).

Candidates for the sodium-independent exchangers at the BBB include organic anion–transporting polypeptide type 2 (oatp2), or BBB specific anion transporter type 1 (BSAT1), which is also a member of the oatp family and is designated oatp14.

Codrugs

Drugs that inhibit a BBB AET could be used as a "codrug" to cause increased brain penetration of a therapeutic drug that is normally excluded from brain by a BBB AET system. For example, AAAD inhibitors are administered as codrugs in conjunction with L-DOPA to optimize brain penetration of the L-DOPA. The discovery of codrugs that inhibit BBB AET systems would be facilitated by the initial cloning of these transporters, followed by their expression in oocytes or some alternative system to enable the development of a CNS codrug discovery program.

Active Efflux (Transport) of Azidothymidine (AZT) Across The BBB

The human immunodeficiency virus (HIV) affects the brain early in the course of the disease that ultimately progresses to acquired immune deficiency syndrome (AIDS). AZT readily crosses the choroid plexus epithelial barrier, which forms the blood-cerebrospinal fluid (CSF) barrier, and enters CSF. However, AZT penetration in the brain parenchyma is minimal, owing to very restrictive transport across the BBB. The AZT model illustrates that drug distribution in the CSF reflects transport across the blood–CSF barrier, not drug transport across the BBB. Drugs may readily enter CSF but might penetrate brain poorly owing to restrictive transport across the BBB.

Biology-Based Approach: BBB Receptor-Mediated Transport

Certain endogenous large-molecule neuropeptides such as insulin, transferrin, or leptin access the brain from blood via receptor-mediated transport (RMT) across the BBB. This transport is mediated by specialized ligand-specific receptor systems, including the insulin receptor (IR) or the transferrin receptor (TfR),
which are highly expressed on the capillary endothelium of brain. Certain peptidomimetic monoclonal antibodies (MAbs) bind to exofacial epitopes on the BBB receptors.

These epitopes are spatially separated from the endogenous ligand-binding site, and the binding of MAbs to the BBB receptor enables RMT of the peptidomimetic MAb across the BBB in vivo. These peptidomimetic MAbs may be used as "molecular Trojan horses" to ferry large-molecule drugs (e.g., recombinant proteins, gene-based medicines) across the BBB.

**Blood Brain Barrier Transport Of Nonviral Gene Medicines**

The surface of the liposome is conjugated with 1000–2000 strands of 2000-Da PEG to form a "pegylated" liposome. DNA encapsulated in pegylated liposomes is stable in blood and has a prolonged blood residence time. However, the pegylated liposome is relatively inert and is not taken up by brain. Therefore, the tips of 1–2% of the PEG strands are conjugated with a peptidomimetic MAb. The conjugation of this molecular Trojan horse to the pegylated liposome forms a pegylated immunoliposome (PIL).

**Gene Therapy of Brain Cancer**

Human U87 glioma cells injected into the brain of severe combined immunodeficiency (SCID) mice leads to the development of intracranial brain cancer. The human cancer was perfused by blood vessels of mouse brain origin. In order to deliver a therapeutic gene to this cancer, it was necessary to traverse two barriers in series: the mouse BBB, and the human tumor–cell membrane. For gene delivery across the mouse BBB, a rat MAb (8D3) that binds to the mouse TfR is used. Gene delivery to human cells is accomplished with a murine MAb (83-14) that recognizes the human insulin receptor (HIR). Thus, the PIL was doubly conjugated with both the 8D3 and 83-13 Mabs. With this system, gene therapy of brain cancer was possible with an intravenous injection of a nonviral formulation. The delivery of a gene encoding antisense RNA to the human EGFR caused a 100% increase in survival time—twice as long as those tumor-bearing mice receiving PIL expressing a control gene (luciferase).

**Gene Therapy Of Experimental Parkinson Disease**

One animal model of PD involves the injection of the neurotoxin 6-hydroxydopamine into the medial forebrain bundle of rats. This toxin disrupts the dopaminergic pathway between the substantia nigra and the striatum, and the subsequent expression of striatal tyrosine hydroxylase (TH) is almost completely blocked ipsilateral to the toxin injection.

A nonviral expression plasmid that encoded rat TH was encapsulated in PILs and targeted to rat brain by a murine MAb (OX26) that binds to the rat TfR. Owing to the presence of the TfR on both the BBB and the neuronal cell membrane, the OX26-targeted PIL carrying the TH gene was delivered across both the BBB.
and the neuronal plasma membrane. With this approach, intravenous nonviral gene therapy caused a 100% normalization of striatal TH activity in the 6-hydroxydopamine-lesioned rat.

**Global Gene Delivery To The Primate Brain**

Gene delivery to the brain of primates or humans is possible with a peptidomimetic MAb specific for the HIR. The HIR MAb is a highly active transport vector, and the level of expression of an exogenous gene, luciferase, in the primate brain targeted with the HIR MAb is 50-fold higher than the level of luciferase gene expression in rat brain targeted with a TfR MAb. Virtually every neuron of the brain expresses the exogenous gene because the plasmid DNA was delivered to brain via transvascular route. The neurons of the cortical columns of the occipital cortex or of the cerebellar cortex of the primate brain express the exogenous gene.

Pharmacological effects of gene therapy delivered with the PIL gene targeting technology are possible because there is such a high rate of gene transfection of brain cells with this approach. The normalization of striatal TH activity was possible with the delivery of only five to ten plasmid DNA molecules per brain cell. Each plasmid may then produce many copies of the expressed mRNA, which in turn produces many copies of the protein.

**Gene Therapy of The Human Brain**

The molecular Trojan horse antibodies projecting from the surface of the PIL are visualized by electron microscopy. The only immunogenic component of this formulation is the MAb, and the immunogenicity of the Trojan horse in humans can be reduced or eliminated with genetic engineering and the production of a "humanized" MAb. (Following the genetic engineering, the amino acid sequence of a humanized MAb is 95% human sequence and 5% mouse sequence.)

A genetically engineered chimeric form of the HIR MAb has been produced and has the same avidity for the HIR in vitro or at the primate BBB in vivo, as the original murine HIR MAb. Therefore, the technology is now available for the noninvasive delivery of nonviral gene medicines to the human brain, making it feasible to create adult transgenic patients within twenty-four hours of delivering the therapy.

**CONCLUSION**

The incorporation of BBB drug delivery strategies within the global CNS drug-development effort is virtually nonexistent. Considering the rate-limiting role played by the BBB in the development of nearly all new drugs for the brain, it is difficult to understand why the BBB has been so consistently underdeveloped in both academic and industry laboratories. Even if a pharmaceutical company wanted to reverse this trend, it would be difficult to hire a critical mass of scientists trained in the BBB. This is because there are no academic programs that specialize in BBB transport biology within Departments of Neuroscience or Departments of Pharmacology in the United States.

However, a few Departments of Pharmaceutical Chemistry within Schools of Pharmacy are now building BBB transport biology programs. Given the chronic underdevelopment of BBB transport biology within academic neurosciences, there is no worldwide infrastructure or critical mass of scientists trained in BBB transport biology. This lack of global BBB infrastructure is the single most important factor that will limit the future of brain drug development.
ACKNOWLEDGEMENT

Authors are thankful to Mr. Sunil Sharma, Chancellor and Dr. Sudhanshu Sharma, Chief Mentor Suresh Gyan Vihar University, jaipur for providing necessary facilities.

REFERENCES
