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Towards nanomedicines for neuro-AIDS

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Abstract

Although Highly Active Antiretroviral Therapy (HAART) has resulted in remarkable decline in the morbidity and mortality in AIDS Patients, controlling HIV infections still remain a global health priority. HIV access to the central nervous system (CNS) serves as the natural viral preserve because most anti-retro viral (ARV) drugs possess inadequate or zero delivery across the brain barriers. Thus, development of target-specific, effective, safe and controllable drug-delivery approach is an important health priority for global elimination of AIDS progression. Emergence of nanotechnology in medicine has shown exciting prospect for development of novel drug delivery systems to administer the desired therapeutic levels of ARV drugs in the CNS. Neuron-resuscitating and/or anti-dependence agents may also be delivered in the brain through nanocarriers to countercheck the rate of neuronal degradation during HIV infection. Several nanovehicles such as liposomes, dendrimers, polymeric nanoparticles, micelles, solid lipid nanoparticles, etc. have been intensively explored. Recently, magnetic nanoparticles and monocytes/macrophages have also been used as carrier to improve the delivery of nanoformulated ARV drugs across the blood-brain barrier (BBB). Nevertheless, more rigorous research-homework has to be elucidated to sort out the shortcomings that affect the target specificity, delivery, release and/or bioavailability of desired amount of drugs for treatment of neuroAIDS.

Keywords

HIV; Drug of abuse; Blood-brain barrier; NeuroAIDS; Anti-HIV drugs; Nanotechnology; Magnetic nanoparticles; Cell-based nanovehicles

1. NeuroAIDS and HIV persistency

Nervous system alterations due to direct or indirect effect of HIV infection, collectively known as neuroAIDS, are more often associated with AIDS patients. At least 10% of diagnosed cases complain about one or other kind of neurological illness [1] and further during the disease progression approximately 50% cases demonstrate neuropathological

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signs or symptoms [2]. In the same line, mild to severe neurological alterations are seen in at least 80% autopsies of AIDS patients [1]. Many CNS diseases such as viral and chronic meningitis, HIV-associated neurocognitive disorders (HAND), vacuolar myelopathy, peripheral neuropathies, etc. have been coupled to the neurovirulent effects of HIV [3–4]. Previously it was believed that penetration of HIV into CNS occurs only during the final stage of infection when viral load is higher in the peripheral blood. Nevertheless, virus level during early infection is as good as that of late phase. Therefore, now it is putatively believed that virus may sneak in the CNS from beginning itself [5]. Towards this end, presence of HIV-particles, -proteins, and -DNA in the CNS along with the intrathecal production of anti-HIV antibodies are detected during the early infection [1, 6–7]. HIV may enter into CNS either directly or as “Trojan passenger” via trafficking of infected monocytes, macrophages, and/or T-cells across the tightly junctioned brain microvascular endothelial cells (BMECs) of blood-brain barrier (BBB) [8]. Early infection of HIV in the CNS triggers production of proteins that alter the BBB integrity (e.g. matrix metalloproteinase) [9–10] and influence leukocytes transmigration across this barrier (e.g. monocyte chemoattractant protein-1) [11]. These intensify the HIV infection resulting in degradation of BBB leading to CNS injury. Numbers of viral proteins have been shown to induce the HIV neurotoxicity and associated pathology. Particularly, HIV protein gp120 and Tat have been extensively studied. Both, gp120 and Tat can breach BBB independent of viral penetration and can be toxic across multiple species and cell lines. Several neuropathological features are noticed due to independent treatment of these proteins [8, 12].

Spread of HIV infection and drug abuse are significantly interlinked. Abusive drugs can alter the neuroplasticity and damage the CNS, analogous to what happens during the HIV infection (e.g. loss of dopaminergic neurons). Most importantly, many illicit drugs such as psychomotor stimulants (Amphetamines), opiates (cocaine and morphine), alcohol, nicotine, marijuana, etc. have been shown to promote susceptibility/progression of HIV infections and associated neuropathogenesis [12–14]. This stimulation of neuropathogenesis in drug-addicted AIDS patients can be attributed to the concerted effect of HIV (or its protein) and drugs of abuse on neurotoxicity. For examples, the immunomodulatory actions of opioids induce the expression of μ receptors and other chemokine receptors in monocytic cells resulting in increased HIV susceptibility and stimulation of HIV expression [15–17]. Also, opiates enhance the production of proinflammatory factors like MCP-1, RANTES, IL-6, ROS, etc. in the brain cells such as neurons, astroglia and microglia [18–19]. These exacerbate the preexisting inflammation of neurons due to HIV infections. Additionally, alteration in endogenous opioids level cause disruption of dopaminergic functions which affect the neuro-immunological ability of nervous system to respond against HIV [20–21]. Psychostimulants such as methamphetamine and cocaine have also been shown to disrupt the dopamine level resulting in oxidative damage of neurons [22]. Likewise, alcohol exposure alters the BBB which leads to increased HIV entry and ROS level in the brain via influx of macrophages³¹. Thus, we see that concerted effect of different drugs of abuse and HIV infection result in sever neurobiological alterations.

Implementation of antiretroviral therapies (ART) has come up as respite for AIDS patients. Quite unrealistically, uninterrupted treatment for several years has been theorized for

complete viral eradication with existing ART [24]. Nonetheless, HIV/AIDS still remains unstoppable and incurable. A major hindrance in purge of HIV is its ability to remain latent in subpopulation of infected cells. Latent cells can escape the deleterious effect of ART and immune response as well and persist for long period of time. Latency is mainly established post-integration of viral genome into the host genome where viral gene transcription remains very low, and no or little virus is produced. The intensity of establishment of viral persistence is determined by many factors such as cells life span, their infection susceptibility and proliferative ability, alterations in cellular physiology and/or immunologic controls, etc. Notably, an appropriate stimulus cause reactivation of latent cells and fresh infectious virions is produced periodically [25]. Thus, persistent infection in a subpopulation of cells serves as an enduring reservoir of rebound viremia. Initially it was believed that the memory CD4⁺T cell population is the only latent reservoir for HIV. However, following discovery by Chun et al. that cells of other lineage could also serve as viral reservoirs; subpopulations of monocytes, macrophages, dendritic cells, follicular dendritic cells, hematopoietic progenitor cells, natural killer cells, mast cells, etc. have been reported to maintain HIV latency [26]. Cells of monocyte-macrophage lineage naturally go across the BBB and infect the immuneprivileged CNS. Perivascular and meningeal macrophages, microglia, and the macrophages of the choroid-plexus are the major phagocytic and antigen presenting cells of the monocyte-macrophage lineage in the CNS. Microglia and perivascular macrophages are the major HIV producing cells in the brain and are largely responsible for the HIV associated dementia. It is believed that HIV is disseminated from peripheral circulation to the brain during the process of repopulation of perivascular macrophages and their subsequent trafficking back into the lymph node, after antigen sampling in perivascular space, may cause reseeded of viruses in the periphery. In contrast to perivascular macrophages, repopulation of microglia is very limited and it can persist in brain for years. Viral replication in microglia upon infection is restricted, mainly due to inherent resistance of these cells to HIV infection and host immune response, which lead to infection persistency. Other CNS cells such as astrocytes, neurons, and oligodendrocytes have also been reported to get HIV infection. While productive HIV infection of neurons and oligodendrocytes are yet to be accepted among scientific communities, maintenance of persistent infection in astrocyte has been undoubtedly proven [25, 27]. Since astrocytes are the most abundant CNS cell types, neuroimmune alterations during HIV infection significantly compromise the optimal microenvironment of brain and disrupt the nourishment and functions of neurons leading to neuroAIDS. Thus, CNS serves as one of the major center for HIV persistency and therefore, effective approaches to eradicate CNS persistency of HIV could be valuable steps towards HIV eradication.

2. Problems of neuroAIDS treatments

2.1. Limitations of current treatments

Highly active anti-retroviral therapy (HAART) has been successfully implemented for management and prevention of AIDS progression. Antiretroviral (ARV) drugs recommended by WHO for HAART formulations belongs to seven classes: Nucleotide Reverse Transcriptase Inhibitors (NtRTI), Nucleoside Reverse Transcriptase Inhibitors (NRTI), Non- Nucleoside Reverse Transcriptase Inhibitors (NNRTI), Protease Inhibitors

(PI), Fusion Inhibitors (FI), Integrase Inhibitors (InI), and CCR5 antagonists³². Basically, combinations of three or more class of antiretroviral (ARV) drugs are formulated for HAART regimens. With the proper HAART treatment, plasma viral load can decline below the detection limit and median life expectancy of AIDS patients may also rise by tenfold [29]. In fact, HAART has resulted in remarkable decline in the mortality rate of AIDS Patients during the last decade and it is predicted that 50% of HIV-infected people will cross the age of 50 by 2015 [30]. Undoubtedly, due to HAART, this lethal disease has been transformed into a chronic pathology. Nevertheless, little irregularity or interruption of HAART treatment leads to resurgence of suppressed viral replication and so, challenge of complete restriction or elimination of progression of HIV infections still exists.

A dramatic decrease in morbidity of many AIDS related symptoms is noticed following the HAART treatment. In the same line, occurrence of some of the neuronal disabilities has also been remarkably declined (e.g. HIV associated dementia (HAD) and symptomatic distal sensory polyneuropathy has been reduced to less than 7 and 10 % of affected people respectively) [31–33]. Nevertheless, a concomitant rise in the other form of CNS dysfunction such as minor cognitive impairments/motor disorders has widely been noticed in the patients on HAART regimes. This resulted in an increase in the cumulative occurrence of HIV associated neurocognitive complications. Vivithanaporn et al (2010) reported that during the decades of 1998–2008, at least 25% of HAART treated patients developed one or other neurological syndrome. Thus, burden of HIV associated neurological disorders prevail on larger scale [33]. This reduced efficacy of current HAART regimens for treatment of increased incidence of neuroAIDS can be attributed to many reasons. Firstly, these treatments are not targeted for inflammatory cascades underlying any of the HIV-associated neuronal disorders. Thus, HAART doesn't have direct effect on the HIV associated inflammatory degeneration. Secondly, inadequate transmigration of ARV drugs across the brain barriers minimizes their detrimental efficacy on resting viral loads in the brain hideout. This may result in gradual generation of resistant viral strain against HAART as seen in some of the infected populations [34]. Third, and importantly, root cause of inadequate transmigration of ARV drugs in CNS, and so, the main obstacle towards treatment of neuroAIDS, is attributed to properties of brain barriers that make it ultra-selective permeable for both, endogenous compounds and xenobiotic molecules as well. Thus, most ARV drugs remain impermeable across the brain barriers. Additionally, ARV drug's short half-life and low bioavailability, due to extensive first pass metabolism including gastrointestinal degradation, may also add to their insignificant arrival in the CNS [35–36]. Moreover, emergence of various side effects and cost of HAART may also result in cessation of treatment. Overall, the basic problem of HAART failure in treatment of neuroAIDS lies in the structural and functional complexity of brain barriers.

2.2. Barriers of CNS

The organizational uniqueness of CNS is featured by three structural barriers, namely, the BBB, blood-cerebrospinal fluid barrier (BCSFB), and the Cerebrospinal fluid-brain barrier (CSFB). In particular, the BBB is a very special anatomical feature because they safeguard the brain from the periphery, respectively by means of tightly junctioned brain microvessel endothelial cells (BMECs) and choroid epithelial cells. This tightly packed structure

possesses very low and selective paracellular permeability. In contrast, BCSFB is quantitatively more permeable and CSFB can readily allow reversible diffusion of [37–39].

The BBB functions as the interface that separates the brain parenchyma from the blood stream. It is an extensive, continuous, fenestrationless, and almost impermeable barrier of tightly junctioned BMECs along the capillaries lining throughout the cerebral microvasculature. The tightness of this transendothelial junction is 50–100 folds higher than the peripheral vessels, giving an electrical resistance of 1500–2000 Ωcm^{-2} . This indicates the severity of opposition-intensity to the passage of molecules from entering the cerebral space. The structural sophistication of the BBB is further compounded by persistent and intimate contact of BMECs to other neuronal cells, mainly pericytes and perivascular astrocytes. The integrity of tight junctions is maintained by three main tight junction transmembrane integral proteins- occludin, claudin and junction adhesion molecules and many cytoplasmic accessory proteins, such as zonula occludens, cingulin, 7H6 antigen, etc. Additionally, BMECs possess few pinocytotic vesicles and its mitochondrial content (both quantity and volume) is also high which, respectively, limit the transcytosis and fuel the increased demand of transport activity associated with the endothelial influx-efflux pump [37]. Only selected molecules necessary for ideal functional efficiency of the brain such as certain amino acids, monocarboxylic acids, amines, sugars, purine bases, hydrophilic molecules like O_2 and CO_2 , etc. are actively transported via mechanisms such as carrier mediated transport, fluid-phase endocytosis, receptors- or absorptive -mediated endocytosis [40]. Many substrate specific transporters such as monocarboxylate transport system, glucose transporter-1, insulin receptor, transferrin receptor, ceruloplasmin receptor, etc. are present on the BMECs [41]. Also, certain neurotransmitters and small lipophilic xenobiotics or endogenous molecules up to molecular weight of 600 Dalton can freely diffuse transcellularly across the BBB [42]. However, all these mode of selective permeability and transportation of small lipophilic or other drug molecules across the BBB provide very little or no benefit for the management of most brain diseases. Transportation of small or large drugs in the CNS, in overall, is also critically affected due to functional sophistication of the BBB. Regardless of the drug's ability or inability to permeate the BBB, their active percentage in the CNS remains below the pharmacological significant level in most cases including many ARV drugs. Towards this end, endothelium of the BBB is equipped with large spectrum of influx-efflux receptors/proteins that can actively transport molecules such as nutrients, metabolites, hormones, neurotransmitters, peptides, drugs, etc. in or out of the brain. These transporters have been classified into two main groups, namely, ATP-binding cassette (ABC) transporters and solute-carrier (SLC) superfamily. Major ABC transporters and SLC carriers that affect drug delivery across the BBB are P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), multi-drug resistance-associated proteins (MRPs), organic cation transporter (OCTs), organic anion transporters (OATs), organic anions-transporting polypeptide (OATPs), equilibrative and concentrative nucleoside transporters (ENTs and CNTs), system L-transporters, etc. Many of these carriers have been shown to affect the distribution of ARV drugs across the BBB [43, 44]. An anti-HIV drug may serve as substrate, inhibitor or both for different influx-efflux transporters or, in other words, these transporters possess overlapping specificity for ARV drugs. Such as, Abacavir, an NRTI, is a substrate for ABC transporters, P-gp and BCRP; and simultaneously, it functions as an

inhibitor of other ABC transporters, MRP-1, 2 and 3. Similarly, Nelfinavir, a PI, is substrate for P-gp and inhibitor of BCRP and two SLC superfamily transporters, OCT-1 and 2 [43]. While being a substrate of efflux transporters causes own inaccessibility to the target, as inhibitor of influx transporters it blocks the CNS entry of corresponding useful substrates. Further, as inhibitors of efflux transporters, an ARV drug may influence the targeted delivery-kinetics of corresponding drug substrate and this may be reason for positive or negative drug interaction during successful or failed recipe of a combined ART therapy. BEMCs also possess enzymatic barrier for metabolism of undesirable neuroactive substances recruited through blood. Elevated expressions of various enzymes such as γ -glutamyl transpeptidase, aromatic acid decarboxylase, alkaline phosphatase, etc. are found in cerebral microvessels. Metabolism-dependent luminal or abluminal expression of these enzymes significantly affects the dynamics and kinetics of xenobiotics in the brain [45]. Thus, in order to maintain the brain homeostasis, various structural and functional uniqueness of the BBB allow exogenous molecules at zero level or far below the pharmacological significant amount. Therefore, the BBB may be considered as the primary impediment that prevents drug penetration into the CNS.

3. Experimental models of BBB for neuroAIDS

Pathological evaluation of HIV-associated neurological complications in human is possible only from individuals who succumb to the disorder. This only lead to snap shot of end-stage pathology, while mechanisms that contribute to the complications remains elusive and so their treatment strategies as well [46]. Though various in vitro and in vivo HIV-infection models has evolved and contributed significantly to its prevention during the last three decades, treatment of neuroAIDS, in large, remains mystery. This could mainly be attributed to the lack of a relevant CNS model. Particularly mimicking the BBB, which dynamically responds to the physiological disturbances and leads to progression of neuropathogenesis in many cases, is a challenging task. Nevertheless, various in vitro and in vivo BBB models for neuroAIDS exist and further efforts to reproduce its real-time anatomy and physiology are underway. Many version of in vitro BBB exists, but in large, they are centered on co-culture of endothelial cells with astrocytes or pericytes. The astrocytes-endothelial cells co-culture model is widely accepted and more dominant because astrocyte end foot processes share the basal lamina, an anatomical layer that envelop > 99% of the BBB endothelium [47]. A schematic of astrocytes-endothelial cells co-culture model is depicted in figure 1. Endothelial cells and astrocytes respectively are grown to confluency on top and underside of a porous membrane in a transwell culture plate. Thus culture plate is bi-compartmentalized via transwell where the top and underside of porous membrane with cells mimics the external (peripheral blood side) and internal (brain microenvironment side) surface of BBB respectively. The transmigration efficacy of molecules (e.g. ARV molecules, nanoparticles, etc.) is measured by calculating their apparent permeability from upper chamber (peripheral blood side) to lower chamber (brain microenvironment side). The intactness of this in vitro BBB model is determined by measuring the trans-endothelial electrical resistance (TEER) using microelectrodes. A mean TEER value of ~200 ohms/cm² cell culture insert is considered consistent with the formation of the BBB [48]. While, in our current understanding, the astrocytes-endothelial interactions provide a closer simulation of

BBB, studies show that lack of exposure to the physiological factors from other surrounding cells and tissues microenvironment may lead to unwanted differentiation of ex situ cultured astrocytes and endothelial cells and so that resultant in vitro BBB could lesser simulate the in vivo one. For example, contact of endothelial cells with neural stem cells (not used in in vitro BBB co-culture model) has been reported to be a significant factor that drives the onset of BBB formation. Also, physical and mechanical stimuli, such as shear stress, generated by various kinds of cell to cell contacts are significant pleiotropic modulators of BBB endothelial cells. These deregulation of physiological factors in in vitro co-culture model of BBB could significantly affect the real-time BBB biological features such as profiles of transporters, ligand, etc.. This, in turn, affects response to endogenous or exogenous stimuli and therefore approaches to develop more sophisticated co-culture BBB models are underway. Even though reproducibility of the entire complex functions of the BBB in vitro is beyond our reach, it certainly simplifies our preliminary understanding of the highly sophisticated brain microenvironment at molecular level. Nevertheless, in vivo assessment of in vitro findings is indispensable in order to establish proof-of-concept for new pharmacological strategies. Development of an in vivo model for validation of therapeutics to combat the HIV associated neurodegenerative disorder remains the biggest challenge for scientific community studying neuroAIDS. In contrast to many microbial pathogenesis where rodent models lead to successful therapeutics, a natural small animal model could not be mimicked for HIV so far. This is because HIV is not infectious to rodents. However, using cutting-edge technical advancements, efforts to develop HIV mouse models are underway [47, 49–50]. In fact, transgenic (gp120 and tat) mouse models and humanized immune system mouse models (NOD, NSG, and BRG model) are already in practice. But reliability upon these in vivo neuroAIDS models is still questionable to a greater extent. Applications and caveats of these mouse models have been discussed in depth by Jaeger and Nath [51]. Among the pertinent animal models for neuroAIDS, macaque model system is currently most widely used and putatively believed to be most effective in bridging existing knowledge-gap in the area of HIV neurobiology. Macaques are basically infected either with the SIV or SIV-HIV chimera (SHIV) which are genetically distinct than HIV. Though neuroinvasion and neuropathology caused by these strains of immunodeficiency virus is parallel to HIV, their disease progression and pathogenesis are quite distinct. More importantly, ARV susceptibility and pharmacokinetics of macaque are very different than human, and therefore, HIV and SIV/SHIV response to antiretrovirals is not analogous. For example, in sharp contrast to HIV, SIV possess natural resistance to NNRTIs class of ARV drugs [46, 52]. Owing to these factors, contribution of macaque model systems in the development of therapeutics for neuroAIDS so far has been very limited. Thus, a much better humanized animal model for better therapeutic assessments for neuroAIDS is an urgent need. In this context, mouse seems to be a candidate species whose humanization could be improved within the boundary of economical and biological sophistication for development of humanized animal models.

4. Advantages of nano-scale technology in drug-delivery

Nanotechnology harvests the unique physicochemical parameters of materials at a nanometer size range. Few of the intrinsic properties of nanoparticles such as higher specific

surface area and increased circulation time have shown remarkable potential for their use as novel drug carrier. Also, other properties like biocompatibility, surface charge, hydrophobicity, and crystallinity are among the fundamental considerations for selecting nanoparticles in the field of medicine [53]. The concept of nano-drugs revolves around development of “target-specific, effective, safe and controllable” drug-delivery method which is need of the hour. Basically, drugs, alone or in association/combination with target-specific molecules, are enclosed in or absorbed on nanoparticles for providing better efficacy and lesser side effects [54].

Superiority of the nano-drug delivery methods could be attributed to combinations of its various features. Firstly, a dramatic increase in the bioavailability of drugs can be achieved through nano-drugs or nano drug-delivery carriers. As such, a significant amount of orally administered nano-capsulated drugs (<100 nm) escape the portal blood circulation route avoiding the reticuloendothelial digestion; rather they are passed to systemic circulation via intestinal lymphatic transport resulting in remarkable reduction in the first pass hepatic metabolism which enhance their quantity and duration of bioavailability. Further, because of the ability to freely flow into capillaries and remarkable increase in blood circulation time, nanoparticles can travel to tissues in every nook and corner of the body. The nano-size particles are compatible for easy intracellular uptake and can travel across different physiological barriers such as BBB, stomach epithelial, etc. The increased circulation time and higher cellular uptake of nanoparticles is greatly influenced by their surface charge and hydrophobicity/hydrophilicity (besides size). While coating of nanoparticles with positively charged molecules such as chitin enhances their attachment to negatively charged surface of cells, coating with hydrophilic compounds (e.g. polyethylene glycol, pluronics, etc) circumvent opsonization resulting in longer blood circulation time. The hydrophobic/hydrophilic nature of nanocarriers also affects the solubility of weaker hydrophilic drugs, and thus in turn influences their bioavailability [53]. Moreover, the larger surface to volume ratio of nanoparticles allows higher drug loading and dissolution rate influencing the bioavailability. Additionally, crystallinity of many nanoparticles (e.g. polymers) significantly affects their degradable speed which influences the biological half-life of associated drugs.

Secondly, nano-drugs possess comprehensive advantages in context to the drug release kinetics. The increased specific surface area of nanoparticles enhances the drug loading ability. Higher amount of drugs in nano-carrier results in initial burst release and then followed by a constant slow release, which affect the kinetics and minimize dose frequency. Similarly, crystallinity of materials affects their dissolution characteristics – the amorphous region degrades faster in compare to crystalline region. Thus, release kinetics of associated/combined drug is affected. Furthermore, surface charge and hydrophobicity of nanoparticles or coating materials play significant role in drug kinetics. These properties of nanoparticles greatly affect their molecular composition and so as their degradation rate is influenced [53]. The increased blood circulation time due to hydrophobic coating prolongs the associated drug release. Also, hydrophilic coating reduces the dose frequency of the poor soluble drugs due to their improved solubility [55].

Third and last, the feasibility of selective targeting can be significantly improved by the nano-drugs. This, in turn, can minimize the side effects and improve the drug efficacy. Nano-drugs or nano-carriers can be molded for both, passive and active targeting. The reduced first pass hepatic metabolism and increased blood circulation time of nanoparticles makes them suitable for the purpose of passive targeting. The application of passive targeting efficiency of nanoparticles has been successfully demonstrated in the case of enhanced permeability and retention effect in targeting tumors of enterohepatic circuit and HIV infections of lymph nodes [56]. In active targeting, drugs or carriers are combined with target moieties or vector molecules that can recognize and bind to a specific target site. Thus, direct administration of a drug into an affected organ or tissue can be achieved. Various substances like antibodies, peptides, hormones, polysaccharides, lipoproteins, etc can be used as targeting moieties. Thermal- and pH-sensitive targeting molecules (e.g. N-isopropylacrylamide) and suitable adjuvant can also be part of active targeting nano-drug carriers [54].

5. Functional nanovehicles for prevention and treatment of neuroAIDS

A complete lack of ARV therapies for nearly 40% of AIDS patients and further, ineffectiveness of HAART in treatment of HIV-associated neurological syndromes has molded the neuroAIDS as a consistent global problem. In the wake of fact that more than 98% of small and large drugs are unable to cross the brain barriers, which is believed to be main impediment in the cure of neuroAIDS, several strategies are being experimented to administer the desired therapeutic levels of anti-HIV drugs across those barriers. Transcranial drug delivery (focused ultrasound and microbubble approach to disrupt the BBB), transnasal drug delivery (direct access to CNS from nasal cavity via olfactory neurons and avoid first pass metabolism), disruption of BBB using pharmacological agent (etoposide and cisplatin), hyper-osmotic solutions (mannitol and urea), prodrugs approach (lipidization of ARV molecules and fusion of drugs with cell-penetrating peptides or antibody specific to BBB receptors), and inhibition of ABC transporters (P-gp) are few approaches with potential to deliver ARV drug across BBB [36,57]. However, these strategies have less strength than limitations which restrict their use as common and novel drug delivery method. An effective drug delivery method or diagnostic agent must have systemic administration ability i.e. majority of therapeutic agents should be delivered to the target site while non-target site should get minimal drug exposure. Complying with this notion, practice of nanotechnology in medicine has shown exciting prospect for development of novel drug delivery system. Investigations of various nanocarriers have generated a promising trend for the better ARV drug distribution to the CNS. Schemes of CNS drug delivery using nanovehicles can be broadly classified based on their passive or active targeting ability [36]. Approaches involving the passive targeting can result in accumulation of higher concentration of drug at endothelium of the BBB. This local gradient difference may allow the drug penetration by passive diffusion. Also, trafficking via non-receptor mediated endocytosis (e.g. macropinocytosis) may enhance the cellular drug uptake. Actively targeted drug trafficking can be possible via receptor mediated endocytosis when periphery of nanocarriers is tagged with ligand molecules matching to specific cell receptor. Nanocarriers can also be tagged/loaded with specific efflux transporters inhibitors

or blocking agent which can result in increased drug concentration across the BBB [54]. Several nanocarrier systems such as liposomes, dendrimers, different nanoparticles, micelles, etc. have been intensively explored and approaches for their improvement are under investigation (Figure 2 and Table 1). Recently, applications of magnetic nanocarriers and monocytes/macrophage based nanoformulations (Figure 3) have gained considerable interest for the treatment of neuroAIDS.

5.1. Polymer-based nanomedicines

Acrylic and polyester polymers are the most studied synthetic polymeric compounds as nanocarriers for CNS drug delivery. Poly (butyl cyanoacrylate) (PBCA), an acrylic polymer, have been extensively explored for this purpose. PBCA possess rapid *in vivo* degradation ability which can minimize their longer accumulation and, in turn, can prevent the brain from potential polymeric toxicity [41]. The lipophilic property of PBCA makes it suitable for loading of various kinds of compounds with hydrophilic property and weak/low basicity. Without causing any permanent physical harm to BBB, PBCA nanoparticles are able to deliver an improved amount of ARV drugs in both brain tissues and CSF. Kuo and Chen (2006) reported that the use of PBCA nanoparticles enhance the *in vitro* BBB permeability of ARV drugs zidovudine and lamivudine by 8–20 and 10–18 fold, respectively. In the same study, application of other acrylic polymer nanoparticle, methylmethacrylate–sulfopropylmethacrylate (MMSPM), showed 100% rise in the BBB permeability of zidovudine and lamivudine [58]. Additionally, PBCA and MMPSM coated with PS-80 (a tensoactive agent) were used for the delivery of ARV drugs, stavudine, delaviridine, and saquinavir, in *in vitro* BBB model. It was found that permeability of these three drugs was enhanced by ~12–16 and 4–11 folds with PBCA and MMSPM formulations respectively [59]. Increase in the BBB permeability of acrylic polymers are facilitated by receptor mediated transcytosis which is triggered by binding of apolipoproteins (adsorbed on the surface of polymeric nanoparticles) to the low density lipoprotein receptors on BMVECs. Besides this transcellular pathway, acrylic polymers can also use the paracellular route via reversible disruption of BBB for short period of time. Despite these merits, application of polymeric nanoparticles is restricted because they are not ideal for the delivery of polar/ionic compounds [36]. Furthermore, process of PBCA degradation can produce toxic formaldehyde by-products. Thus, other polymers like polyesters are considered a safer choice for CNS drug delivery. Two polyester, polylactide (PLA) and poly(lactide-co-glycolide) (PLGA) have been approved by United States Food and Drug Administration for human use. These highly versatile biocompatible polyesters are degraded into glycolic acid and lactic acid which are converted into water and carbon dioxide via TCA cycle and eventually eliminated from the body. Importantly, injection of these polyesters induces negligible and transient inflammatory response. Variety of drugs of both hydrophilic and hydrophobic nature can be entrapped on the matrix of PLA and PLGA. In addition, drugs entrapment can be tailored for sustained release for longer time. Surface modifications of these polyester polymers such as PEGylation (attachment of poly(ethylene glycol), agglutinin coating, alginate embedding, etc., have been strongly recommended for delivery of therapeutic dose across the BBB. Both, PLA and PLGA with certain modifications have been shown to be useful for the improved brain delivery of many non-ARV drugs such as dexamethasone, vasoactive intestinal peptide, superoxide dismutase, etc. Study on ARV

drugs by Destache et al (2010) demonstrated that nanoformulations of ritonavir, lopinavir, and efavirenz with PLGA can maintain a sustain peak of about 28 days in mice brain which is limited to only 2 days with free drugs [60]. Similarly, Rao et al (2008) demonstrated that at two weeks post-administration, PLA nanoparticles in conjugation with Tat peptides could result in 800 fold higher level of ritonavir in mouse brain in compare to drug delivered in solution [61]. It should be noted that the cell penetration ability of Tat peptide makes it a natural accessory for carriers used for drug delivery across BBB. Along with synthetic polymers, natural polymers such as albumin, chitosan, alginate, gelatin, collagen, etc. are also being explored as the potential nanocarrier for brain drug delivery⁵³. Al-Ghananeem et al (2010) investigated the potential of chitosan for delivery of ARV drugs via both, intravenous and intranasal route and found a significant improvement in the level of didanosine in brain and CSF of rat [62].

5.2. Dendrimers-based nanomedicines

Dendrimers are basically globular or spheroidal structures made up of controlled repeats of monomer units branched around a central core (Figure 2B). They can be engineered in the size range of 10–100 nm and may contain many reactive functional end groups, which make them potent for drug delivery systems [53]. Dendrimers may also contain internal void spaces. Thus, both encapsulation (in void space) and conjugation (with reactive end groups) of compounds with different polarity can be possible with dendrimers. Though more than 100 types of dendrimers exist, five main classes used for medicinal purposes are: Polyamine amine, Polypropyleneimine (PPI), Phosphorus, Carbosilane, and Polylysine dendrimers. However, Phosphorus dendrimers has never been used for HIV research [63]. Similar to polymeric nanoparticles, dendrimers have been mostly studied for brain delivery of anti-cancerous drug. Nevertheless, Jiménez et al (2010) investigated the potential of 2G-NN16 dendrimers (a Carbosilane dendrimer) in *in vitro* BBB model for delivery of antiviral (HIV) siRNA. This siRNA/2G-NN16 dendriplexes showed permeability across the *in vitro* BBB and caused a significant reduction in the viral replication [64]. Most of the ARV study involving dendrimers in HIV research has been restricted to different cell types such as, macrophages, dendritic cells, MT2 cells, etc. and thus, more *in vitro* and *in vivo* BBB investigations are required before their use for CNS delivery of ARV drugs. The limited application of dendrimers can be attributed to their complex synthesis process and inconsistent and premature drug release kinetics [36]. The drug release mechanism is also not clear, though some report suggest toward transcytosis through the BBB [63]. Additionally, polycationic surface groups of dendrimers proved to be toxic for negatively charged cell membranes resulting in cell death.

5.3. Micelle-based nanomedicines

Micelles are self-aggregated assembly of amphiphilic molecules dispersed in aqueous media. The diameter of micelle particles may vary from 1–50 nm [53]. Particles are assembled in such a way that there is an inner hydrophobic core and the hydrophilic heads of amphiphilic molecules are exposed outside (Figure 2C & D.). The inner core serves as the encapsulation space competent for the better solubilization of poor water-soluble and lipophilic compounds. Three types of amphiphilic molecules, namely, block-copolymers, surfactants and polymer-lipid conjugates are used for formation of micelles [65]. However,

pluronic block-copolymers has been the most studied micelles types for CNS drug delivery. Pluronic micelles demonstrate zero toxicity to the BBB and can inhibit efflux transporters such as P-gp, MDR1, etc which, in turn, increase their substrate permeability. Notably, many ARV drugs are substrates for efflux transporters/receptors of BBB. Thus, pluronic micelles can serve as both, drug carrier and efflux inhibitor and have been demonstrated to be valuable for CNS delivery of ARV drugs. Batroková et al (1999) showed that exposure of pluronic P85 enhance permeability of ritonavir across *in vitro* BBB [66]. Similarly, *in vivo* experiment by Spitzenberger et al (2007) demonstrated that administration of pluronic P85 alone or in combination with ART (zidovudine, lamivudine, and nelfinavir) resulted in 78–92% reduction in the p24 expressing monocyte-derived macrophages (MDM) from mouse brain in compare to 62 % of only ART treated group at two weeks post-inoculation of HIV [67]. Additionally, Sharma and Garg (2010) suggested that micelles may be tailored for highly selective active targeting by tethering hydrophilic block to ligands specific to HIV reservoir receptors such as lectin [68]. Nevertheless, instability of the non-cross-linked pluronic micelles remains a matter of concern because it may reduce the circulation time resulting in premature drug release.

5.4. Liposome-based nanomedicines

Liposomes are the first and probably the most applied drug delivery carrier [65]. They can be defined as auto-spontaneously arranged unilamellar or multilamellar, spherically closed colloidal vesicles made up of amphipathic phospholipid bilayer membranes surrounding an aqueous core. While one hydrophilic head of phospholipid bilayer is exposed to outside, the other is in contact with vesicle core (Figure 2E). Thus, hydrophobic group of the bilayer is protected from the aqueous environment. This unique character of liposomes allows loading of both hydrophilic (encapsulated in aqueous core) and hydrophobic/lipophilic (incorporated into the bilayer of phospholipid) compounds [69]. Depending upon the processing methods and constituent, size of liposomes may go up to μm ; however, it can be restricted to a minimum of 20–30 nm which is ideal for a nanocarrier [36]. Surface of liposomes can be engineered for active targeting by applying surface charge modifications and/or conjugation of antibodies/ligands specific to diseased cells or tissues including brain and CNS. Additionally, modifications such as PEGylation can improve the inherent poor stability of conventional liposomes and can also reduce their uptake by reticuloendothelial system resulting in improved plasma circulation time [56]. Lipid composition may also be tweaked for better stability and circulation rate. Different types of liposomes used so far for the delivery of anti-HIV/AIDS drugs can be broadly categorized into ionic-, immune- and sterically-stabilized- liposomes [70]. The rationale of using liposomes for ARV drugs is based on the fact that mononuclear phagocytic system recognizes conventional liposome as foreign body; and since monocytes and macrophages are HIV reservoirs and can travel to brain, an improved efficacy of drugs can be achieved [71]. Kim et al (1990) demonstrated that half-life of intraventricularly administered, liposome-encapsulated zalcitabine in the brain of Sprague-Dawley rat increase to 23 h as compared with 1.1 h for the unencapsulated drug [72]. In the same line, Omar et al (1995) showed that liposomal encapsulation could enrich the rat brain with about 13 times more foscarnet – a salvage therapy for multi-drug resistant AIDS patients – in compare to its solution [73]. Further, the superiority of CNS targeting ability of liposomes-loaded AZT-myristate (prodrug of AZT) was studied by Jin et

al (2005). It was shown that, with about 98% encapsulation efficiency and longer half-life, a higher concentration of AZT was found in the brain and other organs of rats [74]. Potential of liposomes have also been evaluated for management of HIV-related opportunistic infections which is critical for the HIV/AIDS patients. Several fold increase in the concentration of amphotericin B (drug for fungal infections in HIV patients) was demonstrated when liposomes tethered with RMP-7 (Bradykinin B2 receptor agonist) were used for delivery across *in vitro* rat-BBB model [75]. Despite these demonstrations of potential of liposomes for improving ARV drug delivery, stability and leakiness of loaded drug during storage remain the issue to be sorted out [53]. Additionally, low drug entrapment ability, especially for water-soluble drugs due to tiny space of aqueous core, is an area for improvement in liposome-based drug delivery.

5.5. Solid lipid nanoparticles (SLN)-based nanomedicines

Recently, SLN (Figure 1F) has emerged as novel particulate system with tremendous potential to be used as a drug delivery nanocarrier. For the synthesis of SLN, one or more biocompatible solid lipids such as fatty acids, glycerides, waxes, glycerine mixtures, etc. are liquefied by heating and dispersed and stabilized in either ionic or non-ionic surfactant which can be emulsifiers and/or co-emulsifiers [69, 76]. The size of resulting solid lipid particles may vary from 1 to 1000 nm and are compatible for carrying both hydrophilic and lipophilic drugs [76]. Because of the lesser non-specific cell toxicity, superior physical and biological stability, high tolerability, higher drug entrapment efficacy, and cost-effective manufacturization, the SLN is believed to be a better nano-drug carrier than other colloidal carriers such as liposomes, PLGA, etc. [36]. Additionally, flexibility to modify its size and charge can be employed for the site-specific targeting and for drug release in response to specific stimuli such as temperature, pH, etc. Also, the immediate burst drug release profile of conventional SLN (attributable to their larger surface area) can be modified for prolonged drug release [53]. All these properties in conjugation with the natural ability of small lipophilic material to cross the BBB make SLN a favorable nanocarrier for the CNS drug delivery. In this context, few studies have shown the potential applicability of SLN for ARV drug delivery across BBB. Kuo and Su (2007) used *in vitro* BBB model of human BMECs and demonstrated that the permeability coefficient of stavudine, delaviridine and saquinavir loaded on SLN was respectively 4–5, 8–11 and 9–11 times as compared with free drugs. In the same study, delaviridine and saquinavir loaded on SLN showed enhanced permeability than those loaded on MMSPM; however, it was suggested that the particle size of these nanoparticles may have significant influence on their drug -loading, -entrapment and BBB permeability efficacy [59]. Further, the same group performed that under the influence of 5mV electromagnetic force (EMF) the *in vitro* BBB permeability of SLN loaded saquinavir was better than that loaded on PBCA and MMSPM; thus, a combination therapy, involving SLN with EMF, was recommended for the beneficial clinical application [77]. Other *in vitro* BBB model study by Chattopadhyay et al (2008) showed a significantly improved cellular uptake of SLN loaded atazanavir in compare to aqueous solution. Similarly, higher cellular accumulation of Rhodamine-123, a substrate of efflux transporter P-gp, was also shown in this study. Thus, it was predicted that SLN may either mask or bypass the efflux pump [78]. Despite these early promising *in vitro* data, supportive *in vivo* experiments are yet to be

tested. Thus, more *in vitro* and *in vivo* study are necessary to delineate the authenticity of SLN for the delivery of ARV drugs in brain.

5.6. Magnetic nanoparticles-based nanomedicines

Magnetite (Fe_3O_4) and maghemite ($\gamma\text{-Fe}_2\text{O}_3$) are the most commonly used magnetic nanoparticles (MNPs) in the field of biomedicine (Figure 3A). They have been extensively investigated for target-specific improved drug delivery. The main advantage that makes MNP superior over other counterparts such as liposomes, micelles, polymeric nanoparticles, etc. is that the unique superparamagnetism property can be utilized for simultaneous monitoring and quantitation of their tissue-specific or nonspecific distribution. Thus, techniques like magnetic resonance imaging (MRI) and magnetometry can be applied for, though indirect, measurement of localization of MNPs associated drugs which may help in determining site-specific optimal or suboptimal dosing. Besides, MNPs possess many characteristics essential for a suitable drug delivery nanocarrier. First, synthesis of MNPs is quite easy and it is feasible to produce monodispersed particles at the laboratory. Second, the flexibility in the size of MNPs, ranging from a few up to tens of nanometers, gives opportunity for optimization of sizes as per requirement of the study. It should be noted that the higher surface to volume ratio enhances target-affinity of MNPs in comparison to the micro-sized magnetic particles and can even manipulate and target at the subcellular organelles levels. Third, MNPs can respond to an external magnetic field. Thus, it is possible to “remote control” the movement of drug loaded nanoparticles for target-specific delivery by applying the magnetic force at the exterior of desired site [79]. Fourth, as mentioned above, the MNPs can function as contrast agent for MRI because signal of protons, an essential requirement for MRI, in the periphery of particles is enhanced by the magnetic moment [80]. Fifth, aqueous solutions of MNPs such as Fe_3O_4 perform amphoterism and develop positive or negative charges at the magnetite-water interface in pH-dependent manner [81]. The flexibility in the surface charge allow binding of wide range of molecules either via direct, but weak, ionic interactions to the MNPs [82] or via surface coating or tethering agents. The well-defined and rigid structures of MNPs, with or without coating, further widen the attachment options by acting as a solid binding platform for various ligands [79]. Sixth, in combination with the liposomes, MNPs can also be developed as hybrid nanoparticles called “magnetoliposomes” (Figure 3A) [57]. The liposomal encapsulation of MNP is advantageous in many ways. While drugs attached with MNPs can be encapsulated in the liposomal core, additional free drugs can be supplemented on the phospholipid bilayers and core as well. Thus, per unit loading efficiency of nanocarrier is enhanced. Also, the liposomal encapsulation protects the drug loaded on the MNPs from the biological degradations and increase the circulation time resulting in increased bioavailability. Furthermore, magnetoliposomes can be utilized for the monocytes/macrophage-based nanodrug delivery at the various inflammatory sites including the brain (explained in section 5.7.) [48,83]. The movement of magnetoliposomes or magnetized monocytes/macrophages for targeted drug delivery can be manipulated in the same way as for naked MNPs. Seventh, doses of MNPs within the permissible limit have non-significant safety concerns and are biodegradable [84]. Particularly, it has been suggested that biologically produced nanosized magnetosomes from magnetotactic bacteria, which is predicted to be highly biocompatible, can be utilized in the same way as artificially

synthesized MNPs [85]. Thus, we see that MNPs possess many features required to be molded for nano-drug delivery in target-specific manner.

Literatures suggest that molecules from variety of group such as proteins, enzymes, drugs, etc can be immobilized on the MNPs [81]. Magnetically guided drug targeting has been successfully demonstrated in various pathological cases including brain carcinomas, inflammations, etc. Also, MNPs as imaging agent have been used to diagnose brain related anomalies. However, its application in the field of HIV/AIDS is limited. Recently, our laboratory explored the potential of MNPs for delivery of ARV drugs in the brain [82]. It was hypothesized that active 3'-azido-3'-deoxythymidine-5'-triphosphate (AZTTP) (a nucleotide analog reverse transcriptase inhibitors) may be directly immobilized on the surface of MNPs under the influence of ionic interaction leading the way for magnetic nanoformulations of ARV drugs. It was found that 1:0.2 ratios of MNPs to AZTTP give the best binding efficiency. Most importantly, the inhibition efficiency of MNP bound drug, as determined by suppression of HIV replication, remains comparable to the free drug. This directed us to formulate the magnetoliposomes for ARV delivery across the BBB [48]. The putative representation of AZTTP carrying magnetoliposomes is shown in figure 2A. Again, the anti-HIV effect of drug in this new nanoformulation was preserved with indications of sustained drug release for at least 14 days of experimental period. Now, potential of AZTTP-magnetoliposomes in the treatment of neuroAIDS was evaluated by measuring their migration, under influence of an external magnetic field, across *in vitro* BBB fabricated from layers of BMVECs and human astrocytes. It was found that without affecting the intactness of artificial BBB, permeability of magnetoliposomes bound AZTTP enhanced by approximately three fold in compare to the free AZTTP. Thus, we were able to demonstrate that MNPs or its derivatives could be used to deliver ARV in the brain. Further, this concept could also be merged with monocytes/macrophage-based delivery of ARV drugs in the brain as explained in the section below (Figure 3C). These hybrid forms of MNPs (magnetoliposomes and magnetic-monocytes/macrophages) can reduce the drugs decomposition, clearance and entrapment by reticuloendothelial systems.

5.6.1. Magneto-electric nanocarrier—Magnetic and electric fields – depending on their intensity and frequency – have been shown to exert therapeutic values for several diseases. We already discussed the importance of magnetically guided active drug targeting for neuroAIDS. Similarly, electric field mediated therapies have been applied for the treatment of many CNS related ailments such as pain, movement disorders, epilepsy, muscle stimulation, etc. However, little is known about the therapeutic values of coupling of magnetic and electric fields. Recently we explored the potential of novel magneto-electric nanoparticles (MENPs) for targeted drug delivery and on-demand drug release of antiretroviral drugs across *in vitro* BBB model. MENPs are a subgroup of multiferroic materials possessing strong coupling ability of its magnetic and electric fields at body temperature. Similar to the MNPs, MENPs Possesses adequately high magnetic moments and, therefore, its movement could be “remote controlled” for its effective penetration across the BBB by applying external direct current (DC) magnetic field. Complementarily, unlike MNPs, its inherent non-zero electric property (AC trigger) could be used to controllably enforce the release of the bound drugs via breaking the symmetry of ionic

bonding (charge distribution) between drugs molecules and nanoparticles. The concept of drug release mechanism by this novel magneto-electrostimulation technique has been illustrated in figure 4. We found that under the influence of remote low-energy DC magnetic field ~ 40% of the MENPs-bound AZTTP could translocate across the BBB and this is ~3 times higher than the free AZTTP. Applying magneto-electric field did not alter the integrity of the in vitro BBB. More importantly, application of a very low AC field (44Oe at 1,000 Hz and) resulted in nearly 100% release of bound drugs from the particles. Owing to these extremely low-field magneto-electricity property MENPs enable dissipation (heat)-free mechanism and functional and structural integrity of the drugs and targeted cells remains unaffected. Thus, unlike other nanocarrier where drug release mechanism depend on “uncontrollable” cellular phenomena and pathology-specific responses, MENPs offer unique capability as a field-controlled drug carrier for on demand release after crossing the BBB and could be relevant to the treatment of many other CNS and other diseases [86].

5.7. Cell-based nanomedicines

The inherent migratory potential of inflammatory-response cells (monocytes, macrophages, dendritic cells, neutrophils, lymphocytes, neuronal stem cells, bone-marrow derived mesenchymal stromal cells, etc) towards the zone of inflammation can be exploited for the targeted drug delivery [87]. Although still at the preliminary stage, this relatively newly hypothesized drug delivery strategy own superior therapeutic and diagnostic potential. While cells can be genetically modified for a continuous production of therapeutic molecules, in the context of nanomedicine, drug loaded nanocarriers such as liposomes, magnetoliposomes, polymers, etc are either packaged inside the cell or, in extreme case, attached to the cell surface for the delivery at the specific injury site [57]. Entry of drug loaded nanovehicles in these cells is mediated by cell surface receptors such as mannose, complement, Fc receptors, etc. Thus, coating of nanocarriers with the receptor-specific moieties such as mannose, folate, gelatin, A- protein, RGD peptide, etc complement the recognition by specific cell surface receptors leading to cellular internalization [87]. Factors such as surface charge, size, and shape of nanocarriers also plays vital role in their internalization by cells. For example, it has been demonstrated that absorption of positively-charged nanoparticles by cells is better than their opposite counterparts. At the same time, the drug preservation efficiency of positively-charged nanoparticles in the cell is also superior. Once inside the cell, it is critical to home the drug-loaded cell-carriers at the right site. To this end, monocytes and macrophages have gained considerable attention for delivery of drug across the CNS. These immunocytes possess margination and extravasation properties and can cross the BBB paracellularly in response to brain inflammation. Thus, “Trojan nanocarriers” residing inside these cells can be delivered in the brain (Figure 3C). Uploading of drugs from cellular carriers at the delivered site is an area which is less understood and need to be intensively investigated for advancement of this novel delivery system. In this context, it is believed that feasibility of controlled drug release from cellular carriers may significantly rely on prolonged stay of cell-carriers at the target site, pathology-specific response (change in temperature, pH, etc), exocytosis of drug containing intracellular vesicles, and intracellular Ca^{2+} concentration of carrier cells. Additionally, external stimulus such as mild hyperthermia may also affect the drug uploading from cell-carriers as has been shown for anti-cancer therapy [88]. Other area which must be addressed

for better practicality of cell-mediated nanocarriers is the minimization of possible cytotoxicity. Mononuclear phagocytes recruited in response to inflammatory cytokines produce reactive oxygen species. In the same line, inhibition of recruitment of monocytes/macrophages in the zone of inflammation is part of therapeutic strategies for many neurodegenerative disorders. Nonetheless, few reported studies to date indicate no shed of cytotoxicity in macrophage-mediated drug delivery in the brain [89]. Eventually, successful clinical application of this method will depend upon loading of nanoformulated drugs either to harvested mononuclear phagocytes from peripheral blood or to artificially differentiated monocytes from harvested stem cells from bone marrow and their re-infusion/infusion to patients [87]. Injection of nanoformulations, coated with monocytes/macrophages specific receptors, in blood circulation may also be another way for development of cell based delivery in clinical settings [90]. However, sufficient research-homework is required before practical application of these speculations.

Cell mediated delivery of nanoformulated drugs is gaining significant consideration for the treatment of various brain diseases, specifically in chronic pathologies such as Alzheimer's, Parkinson's, brain cancer, epilepsy, etc. Its implications in HIV related neuropathogenesis has also shown encouraging trends. Dou et al (2009) demonstrated that macrophage-based nanoparticle platform can successfully deliver the active ARV drug in the brain. Indinavir formulated in suspensions of lipid nanocrystals were packaged into *ex vivo* cultivated bone-marrow-derived macrophages and injected intravenously into severely combined immunodeficient HIV-1 encephalitis (HIVE) mice. High drug release in different regions of the brain were noticed consistently for at least two weeks and corresponding reduction in HIV replication were observed in the HIVE brain regions [91]. In the same line, Nowacek et al (2010) nanoformulated a combination of atazanavir, efavirenz, and ritonavir in a mixture of block copolymers and liposomes and loaded into MDM. *In vitro* drug release study demonstrated their continuous presence from minimum of 15 days to more than 20 days with complete suppression of viral infection [92]. Basic of this study was recently expanded with another nanoformulated combination of atazanavir, ritonavir, indinavir, and efavirenz. By co-cultivating the drugs-packed mononuclear phagocytes with human brain microvascular endothelial cells, it was suggested that intracellular crosstalk may facilitate transfer of drugs from carrier/donor cells to recipient cells [93]. However, mechanisms of this cell-to-cell transfer have not been explained. Recently, our research group extended the ARV drug delivery potential of magnetoliposomes for cell-based delivery [48, 82]. To this end, treatment of magnetoliposomes resulted in monocyte-magnetization level of more than 90%. Again, similar to that found in the case of magnetoliposomes, BBB permeability of magnetized monocytes under the influence of external magnetic force increased by approximately three fold in compare to the non-magnetic monocytes or magnetic monocytes short of external magnetic influence. Thus, we are one step closer to develop the cell-based magnetic nanocarrier for the ARV drugs delivery, specifically targeting areas which are considered as viral sanctuaries such as brain, lungs, etc. Earlier studies based on development of magnetized cell-mediated targeting for one or other purposes including organelle or area specific drug delivery shows remarkable advancement of the relevant outcome [79]. Adding to these, we hypothesized the development of a novel magnetic nanocarrier for ARV drug delivery across BBB (figure 5). Based on the *in vitro* observations

we assume that, under the influence of external magnetic force, ARV drug loaded magnetic nanocarriers in the form of magnetoliposomes can either directly transport across the BBB or as the “Trojan magnetic nanovehicle” will make a vital part of monocytes/macrophage-mediated drug carriers. As such, magnetic hybrid nanoformulations (magnetoliposomes and magnetized monocytes/macrophages) seem to be more practical nanovehicles for drug delivery to the brain.

5.8. Other promising nanovehicles for ARV drug delivery across BBB

The growing popularity of nanotechnology in recent years has steadily opened the field for variety of nanomaterials. With the same perspective, many nanocarriers have been explored for their possible application in the field of nanomedicine. Two novel polymeric materials, nanoemulsions and lipid nanocapsules (LNC), have been preliminarily investigated for their ability to deliver ARV drugs in brain. Vyas et al (2008) demonstrated that the oral administration of saquinavir loaded *oil-in-water* nanoemulsions to Balb/c mice improved the brain uptake in compare to the aqueous formulation. It was suggested that higher availability of drug in the brain may be result of the higher rate of absorption of drug encapsulated in nanoemulsions [94]. Potential of other polymeric novel nanoparticles, LNC, for the delivery of ARV protease inhibitor (Indinavir) in the mouse brain was evaluated by Pereira et al (2005). It was found that tissue/plasma ratios of LNC loaded Indinavir in the brain of normal (*mdr1a*^{+/+}) or efflux transporter, P-gp deficient (*mdr1a*^{-/-}) mouse increased by 1.9 times on average as compared with Indinavir in aqueous solution. At the same time, ratio of aqueous Indinavir in the brain of *mdr1a*^{-/-} mouse was 21.3-fold higher than *mdr1a*^{+/+} mice suggesting that mechanisms other than, or additional to, P-gp inhibition may influence the higher uptake of LNC loaded drugs [95].

Few other nanocarriers such as gold nanoparticles, silver nanoparticles, aptamers, carbon nanotubes, etc. have also been explored either for their modulated antiretroviral activity or for the targeted drug delivery in the field of HIV research. However, these initial applications are either restricted to different HIV-infected cell types or their applicability in ARV drug delivery across CNS have been derived from other brain related, but directly or indirectly connected to HIV research such as drug of abuse, integrity of BBB, etc. Hence, further *in vitro* and/or *in vivo* investigations can shed light on the legitimacy of their application for ARV drug delivery in CNS.

6. Nanovehicles mediated delivery of anti-abuse drugs for treatment of neuroAIDS

Drug addicted AIDS patients account substantial chunk (one-tenth) of HIV-infected individuals. Addiction of abusive drugs remarkably affects the initiation of HIV infections and expedites the progression of associated pathogenesis. Particularly, neuroimmunological changes due to alterations in reward or relapse pathways associated with drug abuse significantly enhance the progression of neuropathogenesis during HIV infections. Towards this end, last two decades of systematic research on molecular mechanism for addiction therapy emphasize on use of antagonists against different neuronal and non-neuronal receptors involved in the signaling cascades induced by drugs of abuse such as dopamine

receptor antagonist, opioid-antagonist, etc. Nevertheless, majority of drug formulations tested so far in the pre-clinical or clinical experimental settings for treatment of neuroAIDS is restricted to the ARV drugs only. ARV drugs are meant to target HIV replication and have little or no effect on the HIV-associated neuronal disorder. Thus, supplementation of anti-dependence agents with ARV drugs in the treatment regimen of drug addicted individuals at very early post-diagnosis of HIV infection may countercheck the rate of concerted neurotoxicity and disease progression by attenuating the rewarding effects of drug abuse.

The application of nanocarriers for target-specific drug delivery may be extended to all sorts of drugs and diseases. This approach has just commenced for addiction therapy (Bonoïu et al, 2009) and attenuation of concomitant deleterious effects of drug abuse and HIV infection [96]. Only recently, Reynolds et al (2012) reported application of gold nanoparticles mediated delivery of siRNA against galectin-1 (an adhesion molecule) in methamphetamine treated, HIV infected MDM. They showed that stimulatory effect of methamphetamine on galectin-1 gene expression is countered due to siRNA knock down and concomitantly HIV infection is attenuated [97]. However, any similar nanocarriers based study to counter the concerted neurodegenerative effect of abusive drugs during HIV infection is completely lacking. Thus, in our views, nanocarriers based *in vitro* and *in vivo* study must be initiated to deliver various anti-dependence agents across the BBB and subsequently the legitimacy of their use for drug abuse associated neuropathogenesis during HIV infections could be delineated. Moreover, transfer of this strategy in clinical settings may be beneficial for suppression of rate of pathogenesis in drug addicted HIV patients and, in turn, it may add to achieve near-normal life expectancy for treated individuals.

7. Future perspectives

Reduction or elimination of HIV load from their safe sanctuaries such as brain still remains the major limitation for treatment of this pandemic. To this end, nanomedicines have shown tremendous promise and various forms of nanovehicles are in pre-clinical stage for targeted delivery of ARV drugs to the drug-impenetrable viral sanctuaries. Thus, relevant research-homework has to be elucidated more rigorously to sort out the various associated shortcomings of this novel approach in treatment of neuroAIDS. Better structural and physiological understanding of the brain barriers, selection of safe (non-toxic and biodegradable) material for the nanovehicles, development of specific brain cell-types targeting strategies, refinement of multifunctional nanocarriers, development of on-demand drug release strategies, universal formulation schemes for intramuscular, intravenous or oral delivery, and more realistic *in vivo* experimentations are few areas which should be given importance to enhance the feasibility of nanodrugs to treat the AIDS related neuropathogenesis. Additionally, delivery of neuron-resuscitating agents such as exogenous neurotrophins to the affected brain may improve the survival, development and function of neurons. Furthermore, special attention should be given to generation of new strain of resistant virus while using the nanodrugs treatment. Eventually, proper pharmacokinetic and pharmacodynamics studies and large scale manufacturing will shed light for successful application of nanodrugs in more realistic clinical settings, impacting the live of HIV infected patients.

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Abbreviation List

ABC	ATP-binding cassette
ARV	Anti-retro viral
AZTTP	3'-azido-3'-deoxythymidine-5'-triphosphate
BBB	Blood-brain barrier
BCRP	Breast cancer resistance protein
BCSFB	Blood-cerebrospinal fluid barrier
BDNF	brain derived neurotropic factor
BMECs	Brain microvascular endothelial cells
CNS	Central nervous system
CNTs	Concentrative nucleoside transporters
CSF	Cerebrospinal fluid
CSFB	Cerebrospinal fluid-brain barrier
DNA	Deoxyribose nucleic acid
EE	Encapsulation efficiency
EMF	Electromagnetic force
ENTs	Equilibrative nucleoside transporters
FI	Fusion Inhibitors
HA	Human astrocytes
HAART	Highly Active Antiretroviral Therapy
HAD	HIV associated dementia
HAND	HIV-associated neurocognitive disorders
HBMVE	Human brain endothelial cell
HIV	<i>Human immunodeficiency virus</i>
HIVE	HIV-1 encephalitis
IL	Interleukin
InI	Integrase Inhibitors
LNC	Lipid nanocapsules
MDM	Monocyte-derived macrophages

ML	Magneto-liposomes
MMSPM	Methylmethacrylate–sulfopropylmethacrylate
MNP	Magnetic nanoparticles
MRI	Magnetic resonance imaging
MRPs	Multi-drug resistance-associated proteins
mV	Millivolt
NNRTI	Non- Nucleoside Reverse Transcriptase Inhibitors
NRTI	Nucleoside Reverse Transcriptase Inhibitors
NtRTI	Nucleotide Reverse Transcriptase Inhibitors
OATPs	Organic anions-transporting polypeptide
OATs	Organic anion transporters
OCTs	Organic cation transporter
PBCA	Poly (butyl cyanoacrylate)
PBMC	Peripheral blood mononuclear cells
PEG	Poly-Ethyl-Glycol
P-gp	P-glycoprotein
PI	Protease Inhibitors
PLA	Polylactide
PLGA	Poly lactide-co-glycolide
RNA	Ribose nucleic acid
siRNA	Short-interfering RNA
SLC	Solute-carrier
SLN	Solid lipid nanoparticles
TEER	Trans-endothelial electrical resistance
TEM	Transmission electron microscope

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Figure 1.

Astrocytes-Endothelial cells co-culture *in vitro* BBB model: Culture plate is bi-compartmentalized via a transwell porous membrane. The top and underside of this membrane is cultured respectively with tightly junctioned endothelial cells and astrocytes which correspondingly mimics the external (peripheral blood side) and internal (brain microenvironment side) surface of BBB.

Figure 2. Different types of nanoparticles under investigation for the delivery of ARV drugs across BBB. (A) Polymeric nanoparticle. (B) Dendrimer nanoparticle. (C) Polymeric micelle. (D) Nonpolymeric micelle. (E) Liposomes. (F). Solid lipid nanoparticles. (G). Lipid nanoemulsions. (H) Lipid nanocapsules.

Figure 3.

Magnetic Nanoparticles (MNPs) based nanovehicles for delivery of ARV drugs across BBB. (A) Transmission electron microscopy (TEM) of Naked MNPs. (B) Magnetoliposomes: MNPs loaded ARV drugs are encapsulated in liposomes. PEG anchoring on the periphery of liposomes enhances its circulatory stability. (C) Magnetized cell-mediated drug delivery: monocytes/macrophages are loaded with magnetoliposomes. Teathering of RGD-peptide on the periphery of magnetoliposomes induces its uptake by monocytes/macrophages.

Figure 4.

A simplified physics of “on-demand” drug release by magneto-electric nanoparticles (MENPs): Ionic charge present on the MENPs shell influence binding of charged molecules (e.g. drugs) on its surface via ionic bonding which persist in the absence of magneto-electric A.C. field. However, supply of A.C. field triggers the dipole moment uniformly in all orientation of MENPs which breaks the intrinsic pattern of positive/negative charges on atoms. This weakens the existing bonding between drugs and particles. When an A.C. field triggers a moment above the threshold value (more than the ionic bond strength between particles and drugs), a homogenous release of drugs from particles could be achieved.

Figure 5.

Proposed schematic of magnetic nanoparticles based ARV drugs delivery across BBB^{57,98}. Under the influence of external non-invasive magnetic/magneto-electric force, drug loaded magnetic nanocarriers in the form of “magnetoliposomes” can directly transport across the BBB. Also, as the “Trojan nanovehicle” magnetic nanocarrier will make a vital part of monocytes/macrophage-mediated drug carriers which can either naturally or under magnetic/magneto-electric force could go across BBB.

Table 1

A comparison of various nano-delivery systems for transportation of ARV drugs across BBB with respect to their current research standings, limitations, and potential technological improvements

Nanoparticle types	BBB Transmigration Potential	Limitations	Suggested Improvements
Polymers	<ul style="list-style-type: none"> Increased transmigration of ARV drugs across in vitro BBB and mouse model. 	<ul style="list-style-type: none"> Not ideal for the delivery of polar/ionic compounds. Transient inflammation may occur. 	<ul style="list-style-type: none"> Nanocarrier potential of natural polymers should be intensively explored.
Dendrimers	<ul style="list-style-type: none"> Increased transmigration of antiviral siRNA across in vitro BBB. 	<ul style="list-style-type: none"> Complex synthesis process. Inconsistent and premature drug release. Polycationic moieties cause cytotoxicity. 	<ul style="list-style-type: none"> More in vitro and in vivo transmigration assays are essentially required. Toxicity of different neuronal cells must be well defined.
Micelle	<ul style="list-style-type: none"> Increased transmigration and efficacy of ARV drugs across in vitro BBB and mouse model. 	<ul style="list-style-type: none"> Particles are comparatively instable which results in premature drug release. 	<ul style="list-style-type: none"> Brain specific ligand tethering may improve the active targeting.
Liposomes	<ul style="list-style-type: none"> Could be developed as "Trojan nanocarrier" residing in the monocytes/macrophages which naturally transigrate across BBB. Increased transmigration of ARV drugs across in vitro BBB and rat model. 	<ul style="list-style-type: none"> Possess low drug entrapment ability. Inefficient loading of water-soluble drugs. Instability and leakiness of loaded drugs during storage. 	<ul style="list-style-type: none"> Surface charge modifications such as PEGylation can improve stability Brain tissue specific antibodies/ligands conjugation could enhance active targeting.
Solid-lipid	<ul style="list-style-type: none"> Increased transmigration of ARV drugs across in vitro BBB model. 	<ul style="list-style-type: none"> Limited in vivo study to show its lab-to-land transfer ability. 	<ul style="list-style-type: none"> More in vitro and in vivo transmigration study required to authenticate its applicability.
Magnetic	<ul style="list-style-type: none"> Could by hybridize with liposomes as "Magnetoliposomes" which can behave as "Trojan magnetoliposomes" residing in monocyte/macrophage. Externally magnetic force mediated movement helps in escape of nanocarriers' uptake from reticuloendothelial system and accelerates active targeting. Increased transmigration of ARV drugs across in vitro BBB model. 	<ul style="list-style-type: none"> Though many in vivo study show site-specific targeting and lab-to-land transfer ability for non-HIV drugs, the same for ARV drugs are very limited. 	<ul style="list-style-type: none"> More in vivo study based on mouse, rat, or monkey models must be performed.
Cell-based	<ul style="list-style-type: none"> ARV drugs loaded on nanocarriers and their packaging in macrophage (which can cross BBB paracellularly) show increased transmigration and antiretroviral efficacy across in vitro BBB and mice model. 	<ul style="list-style-type: none"> Drug release mechanism from cellular carrier at the delivered site is an area which is less understood. Possible cytotoxicity at targeted area via production of reactive oxygen species by 	<ul style="list-style-type: none"> Transmigration ability of many other inflammatory-response cells such as dendritic cells, neuronal stem cells, etc. may lead to find a better cellular carrier. Tethering of cellular specific receptors to nanocarriers may mimic the cellular

Nanoparticle types	BBB Transmigration Potential	Limitations	Suggested Improvements
		inflammatory-response cells such as monocytes inflammatory-response cells such as monocytes	inflammatory-response cells such as monocytes, macrophages, and in turn cell-based cytotoxicity inflammatory-response cells such as monocytes, macrophages, and in turn cell-based cytotoxicity