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A Review of Brain Drug Delivery: Nanoparticle based drug delivery to improve the efficacy of antiretroviral drug therapy in the central nervous system

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Abstract: A comprehensive analysis of the evolution of brain drug delivery is presented, starting from the initial discovery in 1914 that the blood-brain barrier (BBB) prevented the entry of a syphilis drug called salvarsan into the brain. This barrier has posed challenges in delivering drugs to the central nervous system (CNS), resulting in the predominance of lipid-soluble small molecules among FDA-approved CNS drugs. However, there is potential to modify drugs that cannot cross the BBB to utilize the endogenous carrier-mediated transport system of the BBB. This review critically examines the advantages and limitations of various brain drug delivery technologies. Acyclovir (ACV), a guanine derivative antiviral drug, has been on the market for a long time andis available in various forms for oral, topical, and parenteral administration. Despite being an old molecule, it still holds its ground against newer antiviral agents due to its superior clinical application, which includes the ability to suppress recurrence, minimal drug interactions, and affordability. Although ACV is slightly water-soluble, less permeable, and poorly bioavailable, it has the potential to be an effective antiviral molecule. Over the past decade, more than 100 research works have been conducted to explore physicochemical modifications and novel dosageforms to enhance its potentia.
The delivery of drugs to the central nervous system (CNS) is crucial for effectively managing viral infections. However, the presence of natural barrier structures, such as the blood-brain barrier, poses a significant challenge in allowing anti-HIV compounds to reach this anatomical site. Nanotechnology-based strategies offer promising solutions for enhancing drug delivery to the CNS. These approaches have the potential to extend the circulation of drugs in the body, facilitate their passage across the blood-brain barrier, reduce their removal from the CNS, and enable targeted delivery to specific cells and tissues within the CNS. Additionally, nanotechnology can also facilitate intracellular drug delivery, further enhancing the efficacy of antiviral treatments
Keywords: blood–brain barrier, drug targeting Acyclovir, floating, implant, particulate, vesicular

Introduction

The antiviral drug acyclovir (ACV), a synthetic purine nucleoside analogue, was approved by the Food and Drug Administration (FDA) in 1982. It was the first effective medication for treating a wide range of infections caused by herpes simplex virus (HSV) 1 and 2, varicella-zoster virus (VZV), Epstein–Barr virus, and cytomegalovirus. ACV has remained the most successful treatment for HSV infections since its synthesis and discovery in 1974. It is available in various forms such as tablets, capsules, oral suspensions, topical creams, eye drops, nasal ointment, rectal gels, intravenous injections, intravenous infusions, and powder for infusion solution. ACV is marketed under brand names like Zovirax, Avirax, Virax, Civar, Lovir, and GenRX worldwide. The recommended dosage ranges from 200 mg to 800 mg, taken five times a day. The specific dose and frequency depend on the severity of the infections and the variability in individual responses [1-4].

Despite the availability of new antiviral medications in the field, including its prodrug molecule, it has demonstrated significant positive effects. These effects include highly effective suppression of recurrent genital herpes and HSV shedding, as well as an excellent clinical safety profile, particularly for pregnant women [5]. To date, no unpleasant effects have been reported inthe fetus or newborn. Additionally, the molecule has negligible hazards of drug interactions and is reasonably priced. It can be administered through various routes, making it a successful choicefor antiviral therapy. The mechanisms of its antiviral activity involve competitive inhibition of viral DNA polymerase, incorporation into and termination of the growing viral DNA chain, and inactivation of the viral DNA polymerase [6]. Furthermore, this medication has proven to have minimal toxicity as it does not

interfere with DNA synthesis in uninfected cells [7].

Acyclovir, a guanine derivative, has been categorized as BCS class III by the World Health Organization in accordance with their guidelines. However, some scientists argue that it should be classified as class IV due to its highest dose strength of 800 mg. The molecule exhibits certain drawbacks in terms of its physicochemical and pharmacokinetic properties, including slight water solubility (1.3 mg/mL at 25°C), poor permeability (0.12 × 10−6–2.0 × 10−6 cm/s), short half-life (2.5-3.3 h), and low oral bioavailability (10-20%). These limitations have garnered significant attention from researchers, who are striving to develop modified novel dosage forms that can achieve 100% success in therapy [8]. Recognizing that the physicochemical characteristics of an active pharmaceutical ingredient (API) play a crucial role in its stability, solubility, and permeability, certain scientists have focused their efforts on investigating various properties of ACV, such as ionization, structural and electronic properties, polymorphism and pseudopolymorphism, and compatibility with excipients. These studies have ultimatelyfacilitated the development of suitable modifications for the design of dosage forms [9-11].

The advancement of brain drug delivery technology is primarily driven by the blood-brain barrier (BBB) and its hindrance in the progress of novel drugs for the brain. A staggering 98% ofsmall molecule drugs are unable to penetrate the BBB, as demonstrated by the selective organ uptake of histamine, a small molecule drug with a mere molecular weight (MW) of 111 Daltons (Da), in mice [12].

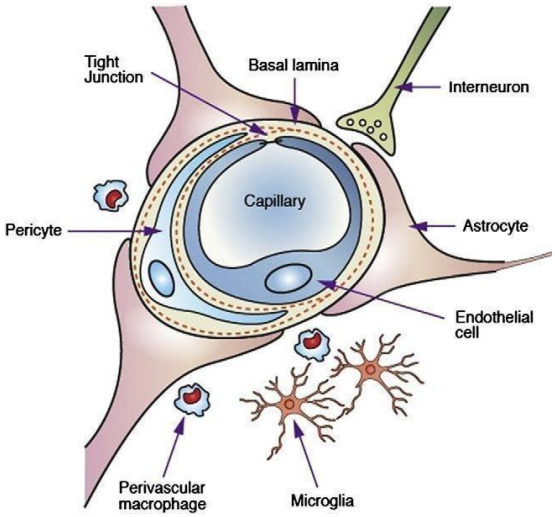

Figure: Diagram of blood–brain barrier (BBB) and other components of a brain.

Invasive brain drug delivery: The advancement of brain drug delivery technology is primarily driven by the blood-brain barrier (BBB) and its hindrance in the progress of novel drugs for the brain. A staggering 98% of small molecule drugs are unable to penetrate the BBB, as demonstrated by the selective organ uptake of histamine, a small molecule drug with a mere molecular weight (MW) of 111 Daltons (Da), in mice [13].

BBB disruption brain drug delivery: The integrity of the blood-brain barrier, which is composed of tight junctions between brain capillary endothelial cells, can be compromised through the administration of harmful substances via intra-arterial infusion or by the introduction of micro-bubbles through intravenous injection, followed by sonication of the brain [14].

Trans-vascular brain drug delivery: The intact blood-brain barrier can be penetrated by modifying the pharmaceutical to interact with various carrier-mediated transporters (CMT) for small molecules or receptor-mediated transporters (RMT) for biologics. This classification also encompasses the advancement of co-drugs that hinder the activity of active efflux transporters (AET) at the blood-brain barrier (BBB), lkm including p-glycoprotein (P-gp), as well as the unrestricted diffusion of lipid-soluble small molecules [15].

Blood–Brain Barrier and Blood–CSF Barrier

The BBB and the blood–CSF barrier are distinct barriers within the brain, both functionally and anatomically. Lllustrates the different anatomical locations of these barriers. The left panel of Figure 3 depicts the BBB, located at the brain microvascular endothelium, while the right panel shows the blood–CSF barrier at the choroid plexus. The BBB, formed by endothelial high resistance tight junctions, effectively prevents solute movement from the blood-to-brain extracellular space through paracellular pathways [16]. Additionally, minimal pinocytosis within the brain capillary endothelium eliminates any non-specific transcellular pathway for solute transport from blood to brain. On the other hand, the blood–CSF barrier is formed by the epithelial cells of the choroid plexus, which line the floor of each of the 4 cerebral ventricles, including the lateral ventricles shown in the right panel [17]. In comparison to the BBB, the blood–CSF barrier is relatively leaky, as indicated by the electrical resistance across these two barriers. The electrical resistance across the endothelium of capillaries within brain parenchyma is estimated to be 8000 ohm·cm2, a value that is 300 times higher than the resistance across the blood–CSF barrier [18]. Due to the increased permeability of the blood–CSF barrier, serum proteins easily pass from plasma to CSF, as evidenced by the high CSF/plasma ratio of IgG, which stands at approximately 0.2%. In contrast, the brain/plasma IgG ratio for the brain parenchyma is less than 0.01% [19].

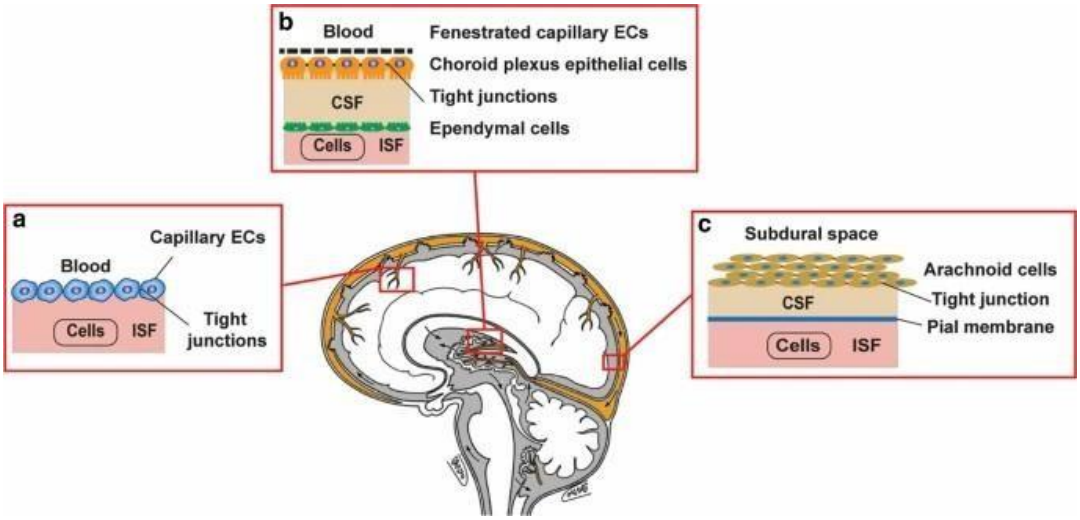

Figure: The brain is safeguarded by various biological barriers, including the blood-brain barrier, blood-CSF barrier, and the arachnoid barrier.

History of the Blood–Brain Barrier

The initial first known of the restricted permeability of blood vessels in the brain was first noted by Ridley in 1695, as discussed by Liddelow and Thakur et al. Ehrlich further demonstrated this restricted uptake of acidic vital dyes by the brain compared to other organs in the 19th century. Through systemic injection of acidic vital dyes in rabbits, it was observed that all organs were stained by the dye except for the central nervous system (CNS) [20]. However, these findings were attributed to the lack of dye adsorption to brain tissue rather than the presence of a barrier between the blood and the brain. In 1900, Lewandowsky conducted experiments involving the intravenous and intrathecal injection of sodium ferrocyanide, as discussed by Liddelow and Macinowski. Lewandowsky observed the effects of ferrocyanide on the CNS following intrathecal injection but not after intravenous administration, and coined the term "blut-thirn-schranke," or blood-brain barrier. In order to assess the selective permeability properties of the cerebral capillaries, it is important to consider the barrier [21]. In 1913,Goldman replicated Ehrlich's findings which demonstrated that the brain did not exhibit staining when acidic dyes were injected intravenously in rabbits. However, Goldman observed that the brain did show staining when the dye was administered intrathecally. These significant findings were subsequently summarized by Mott in 1913 and documented in the English literature. During this period, the prevailing belief was that nutrients from the blood entered the cerebrospinal fluid (CSF) before reaching the brain [22].

The location of the BBB, specifically whether it is in the brain capillary or the choroid plexus, was a subject of ambiguity. Stern, in the 1920s, introduced the term "barrier-hemato-encephalique" or BBB, but

concluded that it was localized to the choroid plexus.However, in the 1940s, other researchers such as Broman in 1941 and Friedemann in 1942 observed that the BBB was clearly situated at the brain capillary wall, not the choroid plexus [23]. Friedemann explicitly stated in his paper that he focused solely on the distribution of substances between blood and the central nervous system (CNS), excluding the distribution between blood and cerebrospinal fluid (CSF). In 1946, Krough noted that Broman had demonstrated that the BBB was localized to the brain capillary endothelium [24].

Consensus regarding the location of the BBB proved to be elusive, as noted by Hassin in 1948. Hassin argued that the cerebrospinal fluid essentially represented the brain's tissue fluids, and suggested that the Virchow-Robin spaces could be considered as the "hemato-encephaliquebarrier" if one were to acknowledge its existence. This viewpoint echoed Mott's earlier perspective from 1913, which posited that the CSF served as an intermediate compartment facilitating the transfer of nutrients from the blood to the brain. Dobbing, in 1961, further contributed to the uncertainty surrounding the BBB's specific location by challenging the notion of a distinct BBB and instead proposed the term "brain barrier system". This concept of a unified "brain barrier system" continues to be employed today, encompassing both the BBB and the blood-CSF barrier as a singular entity [25].

History of Brain Drug Delivery

In 1914, at the dawn of the synthetic pharmaceutical era, the first indication of the BBB posing a challenge in brain drug development emerged. The previous year, Ehrlich introduced salvarsan and neosalvarsan, the pioneering commercial anti-microbial agents marketed by Hoechst for syphilis treatment. Salvarsan consisted of a combination of dimer and trimer complexes of neosalvarsan, an organic arsenical compound with polar properties. The first organo-arsenical compound, atoxyl, was synthesized in 1859 and employed in the treatment of trypanosomiasis. Ehrlich, along with his colleague Hata, determined the structure of atoxyl and subsequently synthesized salvarsan and the more soluble and less toxic neosalvarsan for syphilis treatment. However, it was discovered by Wile in 1916 that the syphilitic spirochete infiltrates the brain, leading to neurosyphilis. Just a year after Ehrlich's publication, McIntosh and Fildes demonstrated in 1914 that salvarsan and neosalvarsan fail to penetrate the rabbit's brain from the bloodstream after intravenous administration [26-29].

- No traces of arsenic can be detected in the brain following the administration of salvarsan and neosalvarsan through intravenous injections in both humans and animals.
- The occurrence is not attributed to a deficiency in the connection between the brain and the drugs, but rather to the drugs' incapacity to permeate the cerebral matter.

Hence, the emergence of the blood-brain barrier and the challenge of delivering drugs to the brain arose in 1914. The inability of neosalvarsan to effectively treat neurosyphilis was primarilydue to its inability to cross the BBB [30-33].

In the 1950s, tricyclic antidepressants and chlorpromazine were created to treat affective disorders of the brain. These drugs were able to cross the BBB through free diffusion due to their high lipid solubility and low molecular weight, they was comparative brain uptake of heroin, codeine, and morphine demonstrated the importance of lipid solubility in BBB transport of smallmolecules. Low-MW, lipid-soluble drugs were effective in treating certain brain disorders, whiledrugs lacking these characteristics were not able to penetrate the BBB. Methotrexate, for example, was developed to treat leukemic infiltration of the meninges but was not effective in the CNS following IV administration. As a result, the drug was directly delivered into the CSF compartment through lumbar CSF injection [34].

The initial brain drug delivery technology, developed by Ommaya in 1963, involved an implantable reservoir for the infusion of drugs into the CSF of a lateral ventricle. Ommaya's intention was to facilitate the long-term treatment of bacterial meningitis with intrathecal antibiotics [35]. However, due to various technical challenges associated with device implantation and maintenance, the adoption of the Ommaya reservoir remained limited. Subsequently, the unintentional development of a brain drug delivery system occurred with the treatment of Parkinson's disease (PD) using L-DOPA, as discussed by Hornykiewicz in 1966. It was understood that PD was characterized by a deficiency of striatal dopamine, and direct administration of dopamine was not effective in treating the condition. However, the administration of L-DOPA, a large neutral amino acid and a precursor to dopamine, proved to beeffective in PD treatment. L-DOPA acted as a prodrug,

undergoing enzymatic conversion into dopamine within the brain through the action of aromatic amino acid decarboxylase (AAAD). The use of L-DOPA as a brain drug delivery approach was serendipitous, as its efficacy was not initially attributed to a blood-brain barrier (BBB) transport mechanism [36-38].

Nearly a decade later, in 1975, Wade and Katzman utilized the Brain Uptake Index (BUI) technique developed by Oldendorf to demonstrate that the uptake of L-DOPA into the brain was facilitated by a BBB neutral amino acid transport system. BBB transport of L-DOPA exhibited saturation and was hindered by other large neutral amino acids. In 1979, a novel brain drug delivery technology was introduced with the objective of transporting drugs to the brain after disrupting the BBB. The infusion of hyperosmolar 25% (1.4 M) mannitol through the intra-carotid artery enhanced the uptake of methotrexate in dogs. In 1982, trans-nasal drug delivery to the cerebrospinal fluid (CSF) was introduced as a strategy to circumvent the BBB. Monkeys were administered progesterone via intra-nasal or intravenous routes, and it was observed that CSF levels of the steroid were higher following intra-nasal administration [39-41].

During the 20-year period from 1980 to 2000, various methods for delivering drugs to the brain were developed. By 1994, trans-cranial approaches had been established, utilizing intra-cerebral implants such as polymers or genetically engineered fibroblasts, as well as convection-enhanced diffusion. Additionally, cationic vectors, including cationized albumin and cationic cell-penetrating peptides (CPP) like tat or penetratin, were also developed. Lipid carriers, such asdocosahexaenoic acid (DHA), were introduced. In 1986, the concept of receptor-mediated transcytosis of receptor ligands through the blood-brain barrier (BBB) was proposed, leading to the subsequent development of monoclonal antibodies (MAbs) targeting either the BBB transferrin receptor or insulin receptor. The model active efflux transporter (AET), p-glycoprotein (Pgp), was identified as having high expression at the brain capillary in 1989. Theapplication of nanotechnology to brain drug delivery began with the introduction of liposomes in1990, followed by nanoparticles in 1995, and dendrimers in 2004. In 2001, BBB disruption through the intravenous administration of microbubbles coupled with focused ultrasound (FUS) was developed, and exosomes were introduced for brain drug delivery in 2011 [42-45].

FORMULATION DESIGNS

Modified release tablets

The successful attainment of release modification is efficiently accomplished in tablets, which serve as the predominant form of dosage for numerous drugs, including ACV.

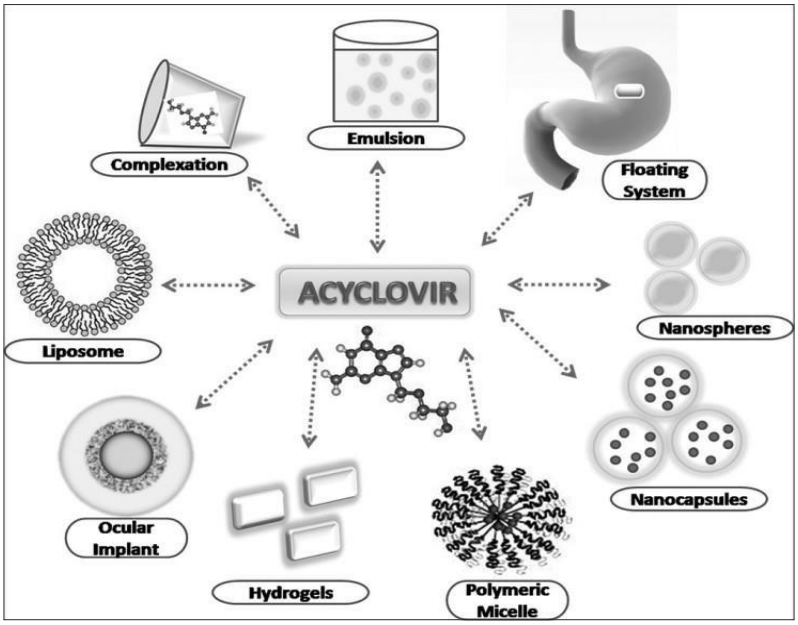

Figure: Novel formulation approaches of acyclovir for antiviral therapy

Consequently, patients readily embrace modifications in such systems. Researchers have extensively documented the diverse range of tablets, categorized as buccal, sublingual, dental, floating, bioadhesive,

Vaginal, rectal, etc., based on the composition of the drug within the matrixor reservoir of the system [46].

Most drugs, including ACV, typically come in a conventional dosage form. However, patients readily accept modifications in these systems. Researchers have described a range of tablet varieties, such as buccal, sublingual, dental, floating, bioadhesive, vaginal, rectal, etc. These variations are based on the type of drug composition in the system, whether it is a matrix or reservoir [47].

ACV oral disintegration tablets were formulated using direct compression and wet granulation techniques, incorporating super disintegrants such as croscarmellose sodium and sodium starch glycolate. The inclusion of sodium starch glycolate in the tablet formulation resulted in remarkable in vitro dispersion time, achieving optimal drug release within a mere 10 minutes [48]

A study was conducted to prepare ACV tablets in a matrix form using the hydrophilic polymer hydroxy propyl methyl cellulose (HPMC) K4M. The tablets were then analyzed to determine the percolation threshold based on kinetic parameters, excipient volumetric fraction at time zero, initial porosity, and water uptake measurements using a modified Enslin apparatus [49]. According to percolation theory, the critical points observed in the dissolution and water uptake studies can be attributed to the percolation threshold of the excipient. This threshold was found to be between 20.76% and 26.41% v/v of the excipient plus initial porosity [50].

A magnetic depot tablet was created as a responsive drug delivery system for oral administration,aimed at extending gastrointestinal transit. To determine the impact of extracorporal magnets, a bioavailability study was conducted on healthy male volunteers. Results showed that when the magnet was placed in the stomach region, the amount of ACV in plasma significantly increased at 7, 8, 10, and 12 hours, with an estimated AUC0-24 of 2802.7 ng/h/mL [51].

Floating delivery systems

Floating systems are highly effective in targeting the stomach and upper portion of the small intestine due to their buoyancy. These systems are particularly beneficial for drugs that have an absorption window in these specific areas. They can be formulated as either single unit or multiple unit dosage forms [52].

The floating capsules of ACV were developed as a single unit dosage form using low density polymers. HPMC K4M was utilized to achieve a zero-order sustained release of the drug. In a separate study, the same group aimed to create floating matrix tablets using similar excipients, along with the addition of Comprito 888 ATO from Gattefosse, France. This addition allowed fordirect compression of the mass and resulted in a zero-order drug release mechanism. Furthermore, other matrix type floating tablets of ACV were formulated using HPMC K100M, HPMC K15M, and natural gums such as locust bean gum, sodium alginate, and xanthan gum. The former two were prepared through direct compression, while the latter three were prepared using the effervescent technique. Additionally, controlled release floating matrix tablets of ACV were designed by combining HPMC K15M CR and polyethylene oxide (Polyox WSR 303). A 32 factorial study was conducted, which revealed that the optimized formulation containing 50 mg of Polyox WSR 303 and 15 mg of HPMC K15M exhibited the best results in terms of drug release and stability [53-55].

Multiple unit floating microspheres were developed utilizing ethyl cellulose through the double emulsion solvent evaporation technique, exhibiting sustained release for a duration of 10 hours and buoyancy for up to 12 hours. Following a similar approach, an ACV-chitosan floating system was also prepared using an innovative lyophilization technique. Emulsion-gelation method was employed to create oil entrapped floating beads, where the percentage of oil playeda crucial role in regulating the floating behavior. The beads, with a drug to polymer ratio of 2:1 and containing 20% oil, demonstrated an optimal entrapment efficiency of 89.54% and sustainedrelease for 8 hours under fed state conditions, with Higuchi model kinetics indicating $n < 0.5$ [56].

In situ gelling systems

The in-situ gelling system has been recognized as a successful method for drug delivery through various routes, such as oral, parenteral, nasal, ophthalmic, rectal, and vaginal administration. This system utilizes temperature-sensitive, pH-sensitive, and ion-sensitive triggers to ensure effective drug release [57].

Several polymers have been identified for this purpose, including Pluronic F127, Gelrite, Carbopol 934P, HPMC K100M, sodium carboxymethyl cellulose, methyl cellulose, xyloglucan, tamarind seed polysaccharide (TSP), sodium alginate, carrageenan, and certain hydrogels. A novel emulgel for ACV was

designed using the former four polymers, which was found to follow non-Fickian anomalous transport of release kinetics [58]. Additionally, an ocular in situ gel was developed using xyloglucan and TSP (0.2-0.8%) with an alginate (0.8%) base, providingaround 75% drug release at 8 hours and enhanced precorneal residence time as proven by gammascintigraphic technique [59].

A new ophthalmic delivery system utilizing a pH sensitive in situ gel with pseudoplastic flow was created and tested for corneal permeation using rabbit cornea in a Franz diffusion model.The in-situ gel had an ocular residence time of 22.4 ± 1.4 min, which was 5.6 times longer than traditional eye-drops (4.0 ± 0.5 min). Additionally, a novel in situ hydrogel system for ocular delivery was developed by incorporating ACV niosomes into carbopol 934 with methyl cellulosecombination gels. The hydrogel released its content within 4-5 hours, followed by sustained release of the niosomes for an even longer period of time [60].

An in vivo experiment was conducted to investigate the impact of carrageenan on a composite thermo-sensitive in situ gel based on poloxamer 407 for vaginal administration. The results ofthe study revealed that the inclusion of carrageenan significantly extended the local residence of ACV and synergistically enhanced the bioadhesive effect of acrylic acid polymers such ascarbopol. This finding was supported by a previous study which examined the effect of pluronic on ACV skin permeation and accumulation using rabbit ear frozen skin in Franz diffusion cell [61].

Implantable delivery systems

A system that can be implanted (either matrix or reservoir type) and allows for the controlled release of medication at the intended location has proven to be highly effective in administering ACV. This method has shown particular success in treating viral infections caused by HSV, particularly in the ocular region when used as ocular inserts, in the vaginal region as vaginal inserts or rings, and also for subcutaneous delivery through implants [62].

Water-soluble polymers such as polyvinyl alcohol and methyl cellulose were utilized in the film casting method to fabricate matrix type ocuserts of ACV. By altering the additives, the rate and drug release profile could be easily adjusted. Another matrix type implant involved dispersing ACV-cyclodextrin complex in HPMC medium and then placing it between cellulose acetate phthalate (CAP) to regulate the drug release rate. This product had a shelf life of 1.8 years and remained stable. The most favorable in vitro release was observed with 5% CAP in the membrane, and in vivo evaluation in rabbits demonstrated a significant IVIVC with the release studies [63].

A soluble ocular insert containing a combination of natural hydrophilic and hydrophobic polymers was utilized to achieve controlled drug release. Additionally, a novel bioengineered corneal implant with ACV loaded silica nanoparticle carriers was created for controlled drug release during corneal transplantation surgery. The sustained drug release from the biosynthetic implants over 10 days was more effective in preventing virally-induced cell death than free ACV incorporated directly into hydrogel constructs. Furthermore, ACV was incorporated into microporous polycaprolactone matrices to design a controlled release intravaginal ring insert for female genital tract viral infections, resulting in a zero-order controlled release of the drug for 30 days and improved antiviral activity using vero cell lines [64].

A subcutaneous delivery system utilizing an implantable silicone device (MED-4050 and MED-4750) was developed to address the reactivation of HSV and VZV infections. The long-term administration of ACV has been shown to decrease the frequency and severity of these infections. In vitro studies demonstrated the device's efficacy in protecting against both viruses, while also providing controlled drug release. The device exhibited an initial burst effect for 5 days, followed by a sustained release period lasting 20-60 days, with an average daily release of 1.4 µg [65].

Vesicular delivery systems

Vesicular systems, such as liposomes, niosomes, and ethosomes, offer a versatile solution for encapsulating hydrophilic and hydrophobic drugs. These systems provide controlled release, leading to improved efficacy and bioavailability. In fact, they have been identified as the preferred choice for designing ACV drug delivery, as ACV is only slightly soluble in aqueous media and has poor permeability across biological membranes [66].

Several studies have reported significant enhancements in drug delivery through the use of niosomes

and liposomes. In vivo oral administration of niosomes demonstrated a twofold increase in bioavailability compared to the free solution, as evidenced by an increase in mean residence time (MRT) in rabbit models. Similarly, the bioavailability of a liposomal mucoadhesive gel administered intranasally was found to be 60.72%, which was comparable to the intravenous route. Furthermore, the ocular pharmacokinetics of ACV encapsulated in liposomes showed significantly higher concentrations in the aqueous humor and a greater area under the curve (AUC) compared to an ointment formulation [67].

Microparticulate delivery systems

Microspheres, microcapsules, nanospheres, nanocapsules, microbeads, co-crystalline particles, and other particulate delivery systems serve distinct purposes and play a crucial role in drug delivery to specific target sites. Various researchers have documented the development of ACV microparticulate systems, as outlined. These systems can be either biodegradable or nondegradable, depending on the type of polymers used in their design (whether natural or synthetic) and the mechanism and location of drug release [68].

Nanoparticulate delivery systems

Nanoparticles (NP) within particulate delivery systems have demonstrated remarkable cellular targeting capabilities and successful permeation into the desired site of action. These systems possess diverse routes of function, which are contingent upon the specific polymers utilized and the design of the dosage form. Additionally, various methods are employed to investigate the physiochemical outcomes of these systems. Several authors have further enhanced the formulations through factorial design, substantiating the efficacy of NP through in vivo animal study models [69].

A novel drug delivery system, utilizing nonpolymeric nanoassemblies, was developed and tested for its pharmacokinetic performance in rabbits. The approach involved the chemical linking of ACV to create an amphiphilic prodrug, which was then formulated into NP using a nanoprecipitation method. The resulting nanoassemblies demonstrated enhanced drug absorptionin tear fluid and aqueous humor when compared to free drug solutions. Additionally, recent research has focused on the synthesis and characterization of five new molecules capable of forming NP in water and effectively encapsulating ACV with high loading and sustained release [70].

The physicochemical properties, sterility, pyrogenicity assessment, biodistribution, and toxicity studies of NP utilized in clinical therapeutics, such as US FDA-approved nanomedicines, were evaluated using diverse analytical techniques.

Bioadhesive drug delivery systems

The adhesive systems available for drug delivery can be categorized into two types: single unit (tablet/capsule) or multi-unit (particulate type or vesicular type) dosage forms. These systems adhere to the mucous lining of biological membranes or surfaces at the specific site of action,

ensuring prolonged drug release. Considering the versatility of ACV administration routes, these systems prove to be highly suitable for targeted drug delivery to specific sites [71].

ACV liposomes were administered nasally using a thin film hydration method with L-α-Dipalmitoylphosphocholine and cholesterol. These liposomes were then incorporated into a bioadhesive system consisting of polyvinyl pyrrolidone (2-6%)/chitosan 2%/carbopol 2%. In vivo studies on rabbits showed a 60.72% bioavailability rate through intranasal administration, which was three times higher than the oral route (15-20%). A similar liposome incorporated bioadhesive hydrogel was created for vaginal delivery of ACV using the polyol dilution method and carbopol 974P resin. The hydrogel was stable, retaining 35% of the drug after 24 hours of incubation. Additionally, an in situ forming mucoadhesive hydrogel of ACV was developed using a novel combination of poloxamers and hyaluronic acid. The hydrogel was analyzed for itsrheological properties, micellar diameter, and mucin adhesion [72].

The development of mucoadhesive microspheres using ethylcellulose and carbopol 974P NF as matrix and polymer, respectively, was achieved. The eggshell membrane was identified as a potential substitute for stomach mucosa in in vitro mucoadhesion measurement. The study showed prolonged residence time in the gastrointestinal tract of rats, resulting in improved bioavailability measured as AUC0−t and MRT of 6055.9 ng/h/mL and 7.2 h, respectively, which were higher than that of ACV suspension. Additionally, mucoadhesive

microcapsules wereprepared using ionotrophic gelation technique with alginate coating [73].

Emulsified dosage forms

Emulsified systems, which incorporate surfactants, have demonstrated distinct benefits in the administration of drugs with low solubility and in concealing the unpleasant taste of active pharmaceutical ingredients (APIs). Proficient individuals have developed various forms of microemulsion, nanoemulsion, and self-emulsified dosage forms to facilitate the controlled release of ACV [74].

A microemulsion formulated with Labrasol and Plurol Oleique as surfactant and cosurfactant respectively, demonstrated increased bioavailability for oral administration compared to commercially available tablets. Additionally, a novel microemulsion-based topical formulation of ACV resulted in complete inhibition of herpetic skin lesions. A liquid-in-oil microemulsion

system containing a 3:2 ratio of tween 80 and span 20 as nonionic surfactants and dimethylimidazolium dimetyhylphosphate as pseudophase was found to have excellent solubility and skin permeation enhancing effects on Yucatan micropig procine skin. The carriers also exhibited low cytotoxicity effects when tested on reconstructed human epidermal model LabCyteTM EPI-MODEL12.

A novel nanoemulsion and self-micro-emulsified drug delivery system was formulated by combining various oils, surfactants, and co-surfactants. This innovative formulation exhibited a remarkable 3.5-fold enhancement in bioavailability when orally administered to male albino rats,surpassing the bioavailability of the pure drug solution [75].

APPROACHES TO BRAIN DRUG DELIVERY

Drug Modification

The BBB permeability of a small drug molecule is closely associated with its octanol-water partition coefficient. Consequently, an increase in the hydrophobicity of the drug may lead to an improved penetration of the modified molecule into the brain. Various modifications can be made to drugs, such as masking or removing hydrogen bonding groups, altering the structure, or chemically linking the active drug with a lipophilic carrier. Many scientists have explored the feasibility of the latter approach, but most of these studies have yielded disappointing results. The lack of significant improvement in the brain uptake of "lipophilized" drugs can be attributed to the decrease in their aqueous solubility, which in turn may lead to,

1. an increase in plasma protein binding,
2. an increase in drug permeability to peripheral tissues.

Explored the impact of plasma proteins on the permeability of the blood-brain barrier forprogesterone, estradiol, testosterone, and corticosterone, which are highly bound to proteins [76].**BBB Disruption**

Methods such as tight junction opening can be employed to manipulate barrier permeability and regulate the delivery of pharmaceuticals to the brain. These approaches to disrupting the blood-brain barrier (BBB) involve the utilization of osmotic or vasoactive agents, surface-active molecules, or organic solvents. The opening of the BBB can enhance the transportation of substances across the brain endothelium through either the paracellular or transcellular pathway. Disruption of the BBB through hypertensive means results in the flux of molecules to the brain that is independent of their molecular weight, potentially due to bulk flow via pinocytosis. Incontrast, hyperosmotic opening leads to the extravasation of FITC-dextran in a size-dependent manner. Furthermore, the administration of hyperosmotic mannitol has been observed to increase the flux of sucrose but not albumin to the rat brain [77].

These studies indicate that transportation to the brain following the opening of the blood-brain barrier (BBB) through osmosis occurs via pores of a specific size, which may suggest the opening of tight junctions. Osmotic BBB opening is achieved by injecting hypertonic solutions such as mannitol or arabinose into the carotid artery. It is believed that the mechanism behind osmotic BBB opening involves a shift in water flux, leading to the shrinkage of endothelial cells. However, recent findings suggest that an intracellular Ca2+-activated complex mechanism may also play a role. The opening of the BBB through osmosis is temporary, as the endothelial cell barrier remains open for approximately 40 minutes before returning to baseline parameters after approximately 8 hours. The use of osmotic BBB disruption has been extensively studied for various applications, including brain tumor therapy. Clinical studies involving brain tumor patients conducted across multiple centers have consistently shown positive tumor responses to chemotherapeutic agents administered

following osmotic BBB opening. Other studies have observed that the infusion of mannitol into the carotid artery results in higher uptake of chemotherapeutic agents in normal brain tissue compared to tumor tissue [78].

Circumventing the BBBIntranasal

It was discovered in the early 1900s that viruses could enter the brain through the olfactory region of the nose. Further studies revealed that certain smaller molecules, such as metals, estradiol, prodrugs, antibiotics, and proteins conjugated with wheat germ agglutinin, could also be transported to the brain and CSF after intranasal administration. However, these molecules must overcome various obstacles in the nasal cavity, including a thick mucous layer and enzymatic barriers. The nasal epithelium secretes numerous enzymes, including peptidases,proteases, and cytochrome P-450 enzymes, with some of the latter being more active in the nasal mucosa than in the liver [79].

Drugs have the ability to reach the brain through various pathways, namely the olfactory nerve pathway, olfactory epithelial pathway, and the systemic pathway via the transmucosal route. The specific mode of transportation depends on the presence of receptors on the olfactory neurons

and the physicochemical properties of the drugs, including their size, lipophilicity, and pKa. In the systemic pathway, drugs are transported through the nasal epithelium and enter the systemic circulation primarily through the respiratory region. Once in the bloodstream, these solutes must cross the blood-brain barrier (BBB) in order to reach the brain. Nasal administration of drugs bypasses the first pass effect, making it an attractive approach for delivering certain drugs to the brain [80].

Interstitial Delivery

Techniques for delivering drugs directly into the brain include injections, infusions, or implants. However, the interstitial delivery method has a major drawback. It has minimal penetration of drugs through the brain parenchyma and the technique is neuroinvasive in nature. To overcome this limitation, implants need to be placed directly at the target site due to the low diffusion of drugs in the brain. Examined the in vivo release and brain deposition of released NGF from polymeric implants. The highest level of NGF was observed in the tissues surrounding the implant, but it quickly declined to 10% at a distance of 2-3 mm from the implant site. In addition to limited penetration, the brain's elimination process can also restrict the brain penetration of drugs. However, creating a pressure gradient can enhance the brain diffusion of various drugs. For instance, the convection flow of molecules in the brain following direct brain infusion has demonstrated significant brain distribution of sucrose and transferrin. Brain implants primarily consist of biodegradable polymeric carriers that contain various drugs or genetically modified cells. These polymeric implants have been extensively studied for their potential use in brain tumor therapy, both in animals and humans [81].

DRUG CARRIERS IN BRAIN DELIVERY

Liposomes in Brain Delivery

Discovered by Bangham in 1961, liposomes have garnered significant attention as carriers for drugs targeting different organs, including the brain (Table I). These spherical vesicles are formed by a phospholipid bilayer in aqueous solutions. They can be categorized into two groups: small unilamellar vesicles (SUV) with sizes below 100 nm and large multilamellar vesicles (MLV) ranging from 100 nm to several microns or even larger. Liposomes have the ability to encapsulate water-soluble molecules within their aqueous core and/or lipophilic drugs within the lipid layer. The composition of liposomes can vary depending on the desired characteristics and formulation procedure, with cholesterol being a commonly used membrane component known tomodulate fluidity, elasticity, and permeability [82].

Nanoparticles (NPs)—Formulation and Characterization

Solid colloidal particles with sizes ranging from 1–1000 nm are known as nanoparticles. These particles can be made up of various materials, including polymers, silica, proteins such as albumin, waxes, or lipids. Drug molecules can be entrapped within the nanoparticle core, embedded in the matrix, adsorbed, or covalently attached to the surface of the carriers. Additionally, the surface of nanoparticles can be modified by attaching cell/tissue-specific targeting ligands.

Nanoparticles have demonstrated the ability to administer drugs in a regulated manner and modify drug

distribution within the body. As a result, these vehicles have been extensively researched for various applications, such as drug delivery systems. Some examples of nanoparticle applications in drug delivery include their use as adjuvants or delivery systems for vaccines, carriers of contrast agents for imaging, or carriers for drugs across the blood-brain barrier. Furthermore, due to their diminutive size, nanoparticles are easily absorbed by numerouscells and are currently being studied for nucleic acid-based drug delivery.

The localization of nanoparticles in vivo is contingent upon the characteristics of the matrix material and surface properties. A significant drawback of nanoparticle technology is their rapid clearance by the reticuloendothelial system (RES). When nanoparticles come into contact with plasma, they may be coated by proteins, opsonins, which are then identified by fixed macrophages as foreign entities that need to be eliminated. Consequently, after systemic administration, the majority of nanocarriers are found in RES organs such as the liver and spleen,which have a high concentration of fixed macrophages. However, this issue can be resolved by modifying the carrier surface with surfactants or poly-(ethylene glycol) (PEG). These hydrophilic surfaces are more resistant to opsonization, leading to an increase in plasma circulation time. The presence of PEG molecules on the surface of many biomedical devices and drug carriers enhances their biocompatibility by reducing protein adsorption and thrombogenicity. PEG chains coated on the surface of nanoparticles increase surfacehydrophilicity and form a steric barrier that protects carriers from plasma protein binding.

There are various methods available for nanoparticle formulation, including emulsion and interfacial polymerization, solvent evaporation, denaturation or desolvation, high pressure homogenization, and the use of microemulsion precursors. The solvent evaporation technique has been widely utilized for the creation of PLGA nanoparticles and microspheres. Typically, polymers are dissolved in an organic phase, which is then incorporated into emulsion or microemulsion systems. Through the process of solvent evaporation, the polymer carriers solidify. However, a drawback of this method is the requirement of an organic solvent, which may leave behind residues in the resulting particles. In the preparation of nanoparticles, the polymerization process has been applied to various polymers such as poly- (alkyl cyanoacrylate),polyacrolein, and poly-(glutaraldehyde). In the formation of polymeric nanoparticles, the monomers are typically contained within the emulsion or microemulsion droplets. Alternatively, the monomers can be suspended or dissolved in the continuous phase. The polymerization process is then initiated either through free radicals or ion formation. Additionally, drugs can be dissolved in the monomer phase or adsorbed onto the surface of preformed nanoparticles. A recent development involves the engineering of novel nanoparticles using warm oil-in-water microemulsion templates [83-85].

Nanoparticles as Drug Carriers Across the BBB

Poly- (butyl cyanoacrylate) nanoparticles (PBCA NPs) have been extensively studied as a system for brain delivery. These nanoparticles are prepared using the free-radical polymerization technique, and drugs are typically adsorbed onto the surface of pre-formed nanoparticles. To enhance their effectiveness, these carriers are then coated with polysorbate 80 (Tween 80). Numerous studies have demonstrated the successful brain delivery of peptides and other drugs when adsorbed to PBCA NPs. Notably, the Leu-enkephalin analogue, dalargin, exhibits high plasma stability but minimal blood-brain barrier permeability and lacks central nervous system action when administered systemically as a peptide solution. However, when dalargin is adsorbed to the surface of PBCA NPs coated with polysorbate 80, significant analgesic effects have been observed in mice. Analgesia was measured by the latency of hindlimb licking in animals placed on a hot plate. The coating of PBCA NPs with Tween 80 has proven to be crucial for the successful brain delivery of dalargin, as control samples without the surfactant coating failed to elicit pharmacological responses. These control samples included blank nanoparticles, dalargin nanoparticles without surfactant coating, peptide solution, peptide solution with polysorbate 80, and a mixture of nanoparticles, dalargin, and polysorbate 80. The findings of these studies have been corroborated by other researchers. Additionally, Schroeder et al. investigated the biodistribution of radiolabeled dalargin in mice following systemic administration of the peptide adsorbed to PBCA NPs, comparing it to dalargin solution and PBCA NPs with dalargin but without the polysorbate 80 coating.

The studies presented in these findings demonstrated that the administration of peptide adsorbed to nanoparticles coated with surfactant led to a notable increase in drug plasma concentration. Of particular importance was the fact that the brain level of dalargin was also significantly higher compared to the control

group. The enhanced brain uptake of dalargin was observed 5 minutes after administration. However, statistical significance was lost at 20 and 60 minutes after injection, and the drug concentration in the brain was similar for both nanoparticle groups, whichwas higher than that of the dalargin solution. This study also demonstrated the feasibility ofusing PBCA NPs as drug carriers across the blood-brain barrier for tubocurarine, doxorubicin, loperamide, and kyotorphin. These drugs typically do not cross the blood-brain barrier under physiological conditions. However, when administered with PBCA-polysorbate 80 nanoparticles, they elicited a pharmacological response in the central nervous system. To gain insight into the mechanism underlying brain delivery of PBCA NPs and their interaction with endothelial cells, in vitro experiments were conducted using cell culture models. Initial reports suggest that nanoparticles are endocytosed, possibly through transcytosis. Incubating PBCA NPs with endothelial cells of various origins resulted in significant cell uptake of fluorescently labeled polysorbate 80-coated nanoparticles compared to uncoated controls, as evidenced by an increase in cell fluorescence [86-88].

Herpes simplex virus (HSV)

HSV infections of the CNS are extremely severe infections that humans can acquire, despite the availability of effective antiviral therapy. There are two distinct types of HSV infections that affect the CNS. The first type is herpes simplex encephalitis, which primarily affects older children and adults. This form of encephalitis is the most common cause of sporadic fatal encephalitis and is almost always caused by HSV-1. The second type is neonatal herpes simplex encephalitis, which occurs within the first month of life and is typically caused by HSV-2. It is worth noting that herpes simplex encephalitis predominantly affects the temporal lobe, leading to clinical symptoms that vary depending on the duration of the disease. This temporal lobelocalization is a defining characteristic of herpes simplex encephalitis in individuals older than 3 months. However, CNS disease in newborns can either be diffuse, resulting from bloodborne transmission, or focal, resulting from neuronal transmission. The annual incidence of bothdiseases is estimated to be around 1500 to 2000 cases [89].

Treatment

Acyclovir is the preferred medication for treating neonatal HSV infections of the CNS and HSE. It is a potent inhibitor of HSV replication and has been a significant breakthrough in antiviral therapy. Acyclovir is a synthetic acyclic purine nucleoside analog that specifically targets and inhibits HSV-1 and HSV-2. The virus-encoded thymidine kinase converts Acyclovir into its monophosphate form, a process that does not occur in uninfected cells. Cellular enzymes then catalyze the conversion of Acyclovir into its diphosphate and triphosphate forms, resulting in much higher concentrations of Acyclovir triphosphate in HSV-infected cells compared to uninfected cells. Acyclovir triphosphate effectively inhibits viral DNA synthesis by competing with deoxyguanosine triphosphate as a substrate for viral DNA polymerase. This leads to the termination of DNA synthesis as Acyclovir triphosphate lacks the necessary 3-hydroxyl groupfor DNA chain elongation. The viral polymerase has a stronger affinity for Acyclovir triphosphate than cellular DNA polymerase, resulting in minimal incorporation of Acyclovir into cellular DNA. In laboratory tests, Acyclovir has demonstrated activity against HSV-1 (with an average 50%-effective dose [ED50] of 0.04 tig/mL), HSV-2 (ED50 of 0.10 lig/mL), and varicella-zoster virus (ED50 of 0.50 Ag/mL) [90].

Acyclovir has recently been found to be equally effective as vidarabine in treating neonatal HSV infections of the brain, without any superiority. Among infants with localized disease in the skin, eye, or mouth, there were no reported deaths. However, the mortality rates were 18% and 55% for infants with encephalitis or disseminated infection, respectively. Interestingly, even though the HSV infection seemed localized, children who received vidarabine and acyclovir developed neurological impairment more than 2 years later, resulting in morbidity in 10% and 2% of patients, respectively. After experiencing encephalitis, 50% of survivors treated with vidarabine and 43% of survivors treated with acyclovir showed normal development. The rates of normal development for infants who survived disseminated infection were 62% and 57% following vidarabine and acyclovir treatment, respectively [91].

In these studies, a dosage of 10 mg/kg of acyclovir was administered every 8 hours for a periodof 10-14 days. Interestingly, unlike other studies that compared vidarabine and acyclovir, both drugs had similar effects on the outcome of the disease. However, infants who received acyclovir experienced faster viral

clearance compared to those who received vidarabine. It is worth noting that around 10% of infants with encephalitis or disseminated disease with brain involvement experienced a relapse within 5-15 days after completing the antiviral therapy course.

Despite treatment of CNS infection, significant morbidity and mortality still occur. In order to improve outcomes, future therapeutic efforts should focus on developing antiviral drugs that are more effective against HSV and can cross the blood-brain barrier. It is also important to prevent the progression of infection to the CNS and the development of disseminated disease. Ideally, preventing neonatal HSV infection through immunization of at-risk mothers or administering immune-prophylaxis and therapy to newborns of mothers with asymptomatic primary or initial infection would be the best approach. While the current recommended dosage is 10 mg/kg three times daily, higher dosages and longer courses of therapy are being studied [92].

Therapy for herpes simplex encephalitis (HSE) that occurs after the newborn age requires the administration of acyclovir at a dosage of 10 mg/kg every 8 hours for a period of 10-14 days. When comparing the effectiveness of acyclovir and vidarabine in treating biopsy-proven HSV, the mortality rates at 3 months were found to be 19% and 50% respectively. However, over time, the mortality rate associated with HSV increased to 30% among acyclovir recipients. Approximately 38% of patients who received acyclovir were able to regain normal function. It was observed that patients with a Glasgow coma score of less than 6, particularly those with encephalitis lasting more than 4 days, had a significantly poorer outcome. Therefore, in order to achieve the best possible outcome, it is crucial to initiate therapy before there is a significant decline in the Glasgow coma score, ideally aiming for a score higher than 10.

The data highlights the urgency for better treatment plans for HSE. The implementation of PCR, as mentioned earlier, in analyzing CSF of patients with suspected HSE can aid in improving our diagnostic abilities. Brain biopsy should only be considered for patients with an unclear diagnosis or those experiencing progressive neurological decline despite acyclovir therapy.

In the future, drugs that possess improved ability to cross the blood-brain barrier, exhibit higher effectiveness against HSV, and have good oral bioavailability for administration after intravenous therapy will undergo evaluation. The issue of clinical relapse continues to pose challenges for patients with HSE, as well as those with neonatal herpes infection. It is estimated that around 5%-10% of patients with HSE will experience clinical relapse [93, 94].

Conclusion and remaining challenges

This review provides a comprehensive overview of the different research areas, ongoing studies, and prospects for the drug. The extensive research conducted on the pharmaceutical aspects of ACV has established it as a promising and versatile molecule for treating HSV infections. Numerous pharmacological studies, involving both animal models and human volunteers, have further supported its efficacy as a therapeutic agent. Additionally, clinical trial reports have provided evidence of its superiority over other medications available in the market. Recent discussions have focused on various formulation approaches, with particular emphasis on nano and micro particulate systems and vesicular delivery methods.

The BBB serves as a protective barrier for the brain, shielding it from the external environment. However, this barrier also poses a challenge for delivering drugs to the brain. Various strategies have been devised to overcome this obstacle, each with its own advantages and disadvantages. The choice of approach depends on the desired therapeutic outcome. Among the most versatile methods is the utilization of nanoparticles and liposomes as carriers for drugs across the BBB. It is crucial to develop carriers that are biocompatible, biodegradable, and do not disrupt the structure and function of the BBB or cause systemic toxicity. The success of using nanocarriersto deliver effective drug doses to the brain relies on the identification of a specific target within the brain that enhances the uptake of nanoparticles and liposomes.

HSV infections of the central nervous system (CNS) continue to be prevalent and cause significant morbidity. Despite treatment with acyclovir, many patients still experience neurological impairment. The development of drugs with better CNS penetration and increased activity against HSV is crucial for improving outcomes. Early diagnosis is also essential, and PCR has been successful in identifying individuals with probable HSE. The use of PCR with proper controls can further clarify the spectrum of HSV infections of the CNS and its correlation with neurological outcomes.

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