Section A-Research paper

Unlocking the Brain's Fortress: Trojan Horse Liposomes as a Revolutionary Approach to Drug Delivery

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ABSTRACT:
The effective delivery of therapeutic agents to the brain is hindered by the blood-brain barrier (BBB), a highly selective and impermeable barrier that restricts drug entry. Researchers have explored various strategies, including Trojan horse liposomes, to overcome this challenge. This comprehensive review provides an in-depth overview of current research on Trojan horse liposomes for brain drug delivery, focusing on advantages, limitations, and future prospects. The BBB's physiology and function are explained to highlight its significance as a barrier. Traditional drug delivery limitations pave the way for Trojan horse liposomes as a potential solution. The review explores liposome formulation, composition, and functionalization, elucidating how they exploit endogenous transport systems to cross the BBB. Considerations in liposome design, such as surface modifications and targeting ligands, are discussed. Research findings on Trojan horse liposomes' efficacy in delivering therapeutics across the BBB using *in vitro*, *in vivo*, and preclinical models are presented, along with specific examples of drugs and diseases targeted. Advantages, such as enhanced drug delivery and reduced toxicity, are analyzed, while challenges like liposome stability and immunogenicity are addressed. Future prospects, including nanotechnology advancements and personalized medicine, are explored. Existing challenges such as large-scale manufacturing and clinical translation are considered. The conclusion emphasizes Trojan horse liposomes' potential for brain drug delivery and underscores the importance of overcoming BBB limitations through continued research efforts.

Keywords: Blood-brain barrier, Drug delivery, Liposomes, Targeting, Trojan

INTRODUCTION

The major challenge in drug delivery to the brain is the existence of the blood-brain barrier (BBB). Capillaries in the brain are lined with specialized endothelial cells that lack fenestrations (pores) and are tightly sealed with junctions, forming the BBB(Daneman & Prat, 2015). This barrier restricts approximately 98% of small-molecule drugs from crossing into the brain, while only minute amounts of large-molecule drugs are able to do so. Additionally, there is the blood-cerebrospinal fluid barrier, which is formed by the epithelial cells of the choroid plexuses(Abbott et al., 2010).

Various techniques have been developed to overcome these barriers and enhance the amount and concentration of therapeutic compounds in the brain. However, the challenges don't end with just crossing the BBB. Even if a compound manages to cross the barrier, it may not reach a therapeutically relevant concentration in the brain. This can be due to the drug's low permeability through the barrier or it's binding to other proteins in the body, which can render it inactive or prevent it from passing through the barrier(Ballabh et al., 2004).

Moreover, enzymes present in the brain tissue can also lead to the deactivation of the drug, even if it successfully enters the brain. These problems must be carefully addressed and considered

when developing drug delivery solutions to effectively target brain tissue. Finding ways to ensure an adequate concentration of the therapeutic compounds in the brain and safeguarding them from inactivation or binding issues are critical aspects of successful drug delivery to the brain(Garcia-Garcia et al., 2005).

THE PRIMARY FUNCTIONS AND ROLES OF THE BBB

The BBB serves several vital functions in protecting and maintaining the brain's well-being (Abbott et al., 2010; Bernacki et al., 2008; Campos-Bedolla et al., 2014):

Protection: The BBB acts as a selective barrier, safeguarding the brain from harmful substances and pathogens present in the blood. It restricts the passage of large molecules, toxins, and pathogens, thus preserving the brain's delicate environment.

Regulation of Brain Homeostasis: By controlling the transport of essential nutrients, ions, and molecules into the brain, the BBB maintains a stable chemical environment crucial for optimal brain function.

Neurotransmitter Regulation: The BBB carefully regulates the entry and exit of neurotransmitters and other signaling molecules, ensuring balanced communication between brain cells (neurons) to avoid excessive excitatory or inhibitory activity.

Maintaining Brain Fluid Composition: The BBB prevents fluctuations in the brain's extracellular fluid composition by limiting the entry of molecules from the bloodstream that could disrupt its balance. This function supports stable neuronal activity.

Protection from Systemic Toxins: While beneficial in most cases, the BBB can pose challenges in delivering medications for neurological conditions, as it hinders many drugs from crossing into the brain.

Immune Privilege: The BBB contributes to the brain's immune privilege, limiting the access of immune cells and certain immune responses. This prevents excessive inflammation and immune-related damage to brain tissue.

To address the difficulty of drug delivery across the BBB, researchers have developed the concept of "Trojan horse liposomes." These liposomes are designed to exploit the brain's endogenous transport mechanisms, allowing them to penetrate the BBB and deliver therapeutic agents directly to the brain. This innovative approach holds promise in improving drug delivery to treat various neurological disorders and conditions.

CONCEPT OF TROJAN HORSE LIPOSOMES

Trojan Horse Liposomes (THLs) are pegylated liposomes designed for targeted gene delivery. They encapsulate supercoiled plasmid DNA using optimized naturally occurring lipids. PEGylation enhances stability, and transferrin molecules on the liposome surface aid targeted delivery to cells with transferrin receptors. THLs offer promising potential for gene therapy and genetic research by selectively delivering therapeutic genes to specific cells while minimizing off-target effects. THLs, also referred to as targeted liposomes or ligand-targeted liposomes, are a specialized type of liposomes engineered to deliver drugs or therapeutic agents to specific cells or tissues in the body. Liposomes are small, spherical vesicles composed of lipid bilayers, resembling cell membranes. They are widely used as drug delivery carriers due to their biocompatibility, capability to encapsulate various drugs and potential for targeted drug delivery.

The design of Trojan horse liposomes involves attaching specific ligands or targeting molecules to their surface. These ligands are chosen to interact with receptors or markers present on the target cells or tissues. By doing so, the liposomes can selectively bind to and enter the desired cells, enhancing the drug's delivery precisely to the intended site while reducing exposure to non-target tissues. This targeted drug delivery approach holds promise in improving treatment

efficacy and reducing potential side effects, making it an area of active research in drug delivery and therapeutics.

Endogenous transport mechanisms to penetrate the BBB

Trojan horse liposomes leverage endogenous transport mechanisms to facilitate their passage through the blood-brain barrier (BBB) and deliver therapeutic agents to the brain. Inspired by the ancient Greek myth of the Trojan horse, where soldiers hid inside a wooden horse to infiltrate the city of Troy, Trojan horse liposomes are designed to "conceal" drugs or therapeutic agents within their lipid bilayers and exploit the BBB's transport mechanisms to gain entry into the brain(Pardridge, 2012).

THE DESIGNING OF TROJAN HORSE LIPOSOMES

The design of THL is described below (Hathout et al., 2019; D. Jiang et al., 2020; Pardridge, 2020; Yan et al., 2022).

Liposome Formulation: Liposomes are spherical vesicles composed of lipid bilayers that can encapsulate both hydrophobic (lipophilic) and hydrophilic drugs within their core and aqueous interior, respectively. This characteristic allows them to carry a wide range of therapeutic agents effectively.

Surface Modification: To target specific transport mechanisms at the BBB, the surface of the liposomes is modified with ligands or molecules that can interact with receptors or transporters present in the endothelial cells of the BBB. These ligands may include antibodies, peptides, aptamers, or other molecules known to interact with BBB transporters or receptors.

Exploiting Receptor-Mediated Transcytosis: One of the key endogenous transport mechanisms targeted by Trojan horse liposomes is receptor-mediated transcytosis. In this process, certain molecules (ligands) bind to specific receptors on the surface of BBB endothelial cells. The liposomes are designed with these targeting ligands on their surface. As they circulate in the bloodstream, the liposomes encounter the BBB and bind to the corresponding receptors through the ligands.

Transcytosis and Drug Release: Once bound to the receptors, the liposomes are internalized by the BBB endothelial cells through receptor-mediated endocytosis. This process forms vesicles containing the liposomes and their encapsulated therapeutic payload. These vesicles then undergo transcytosis, transporting the liposomes and their cargo across the endothelial cell.

Release in the Brain: After transcytosis, the vesicles fuse with the endothelial cell membrane on the brain side of the BBB, releasing the liposomes and their drug payload into the brain's extracellular fluid. From there, the therapeutic agents can diffuse to the target cells within the brain and exert their therapeutic effects.

By utilizing receptor-mediated transcytosis and other endogenous transport mechanisms, Trojan horse liposomes can effectively bypass the restrictive nature of the BBB and deliver drugs or therapeutic agents to the brain. The incorporation of targeting ligands on the liposome surface enhances their specificity, allowing them to selectively interact with the BBB endothelial cells and gain access to the brain parenchyma. This targeted drug delivery approach has the potential to improve the efficacy of treatments for neurological disorders and minimize side effects by reducing exposure to non-target tissues.

WORKING OF TROJAN HORSE LIPOSOMES

The working of THL is summarized as follows (Hathout et al., 2019; D. Jiang et al., 2020; Lee et al., 2020; Yan et al., 2022).

Liposome structure: The fundamental structure of liposomes comprises a lipid bilayer that allows for the encapsulation of both hydrophobic (lipophilic) drugs within its core and

hydrophilic drugs within its aqueous interior. This characteristic versatility enables liposomes to serve as effective carriers for a wide range of therapeutic agents, including chemotherapy drugs, genes, peptides, and imaging agents.

Targeting ligands: To impart targeting capabilities, specific molecules known as ligands are attached to the outer surface of liposomes. These ligands can include antibodies, peptides, aptamers, or other molecules with a high affinity for receptors or antigens present on the surface of the intended target cells or tissues.

Targeting and delivery: Upon administration into the body, Trojan horse liposomes circulate in the bloodstream until they encounter the target cells or tissues. Liposomes equipped with the appropriate ligands selectively bind to the corresponding receptors or antigens on the target cells, facilitating their specific uptake into the cells through a process called receptor-mediated endocytosis.

Payload release: Once inside the target cells, the liposomes may undergo fusion with the cell membrane or be internalized into endosomes. Subsequently, they release their therapeutic payload either through passive diffusion or in response to specific intracellular conditions, such as changes in pH or enzymatic activity.

The concept of Trojan horse liposomes thus exploits these mechanisms to deliver therapeutic agents precisely to the target cells, enhancing treatment efficacy and minimizing off-target effects. This targeted drug delivery approach holds significant potential in advancing medical treatments for various diseases, particularly those requiring localized and precise drug delivery.

ADVANTAGES OF THL

THL offers several advantages as a promising approach for brain drug delivery (Choi et al., 2012; Collet et al., 2013; Haranath, Reddy, et al., 2022; Li et al., 2020):

Enhanced BBB Penetration: The unique design of Trojan horse liposomes allows them to exploit endogenous transport mechanisms, such as receptor-mediated transcytosis, to effectively cross the blood-brain barrier (BBB). This enhanced penetration enables therapeutic agents to reach the brain, which is otherwise challenging with conventional drug delivery methods.

Targeted Drug Delivery: Liposomes can be surface-modified with specific ligands that have an affinity for BBB receptors or transporters. This targeting strategy allows Trojan horse liposomes to selectively interact with BBB endothelial cells and deliver therapeutic agents precisely to the desired brain regions, reducing off-target effects.

Versatile Drug Encapsulation: Trojan horse liposomes have the ability to encapsulate both hydrophobic and hydrophilic drugs within their core and aqueous interior, respectively. This versatility allows them to carry a wide range of therapeutic agents, including chemotherapy drugs, gene therapies, peptides, and imaging agents.

Reduced Systemic Toxicity: By targeting drug delivery specifically to the brain, Trojan horse liposomes can minimize systemic exposure to the therapeutic agents. This reduces the risk of toxicity and side effects in non-brain tissues, improving the safety profile of treatments.

Improved Therapeutic Efficacy: The enhanced delivery of therapeutic agents to the brain through Trojan horse liposomes may lead to increased drug concentration at the target site, enhancing the therapeutic efficacy for neurological disorders and brain-related conditions.

Potential for Combination Therapies: Trojan horse liposomes can be engineered to carry multiple therapeutic agents simultaneously. This capability opens the door for combination therapies, where different drugs can work synergistically to address complex diseases or conditions with multiple underlying factors.

Theranostic Applications: The development of theranostic liposomes that combine drug delivery with diagnostic imaging agents could revolutionize neuroimaging and treatment monitoring. These liposomes enable real-time monitoring of drug distribution and therapeutic response in the brain, allowing for personalized treatment adjustments.

Nanotechnology Advancements: Advancements in nanotechnology and liposome engineering may lead to the development of more sophisticated liposomal formulations with improved stability, release kinetics, and BBB penetration capabilities, enhancing their overall effectiveness.

MECHANISMS OF BBB PENETRATION

The main mechanism involved are as described below (Ahad, Chintaginjala, et al., 2021; Pardridge, 2010, 2020; Xia et al., 2008).

A. Receptor-Mediated Transcytosis:

Absolutely, you are correct. While most large molecules are unable to cross the BBB due to their highly selective nature, some larger molecules, such as insulin and transferrin, can traverse the BBB through a process known as receptor-mediated transcytosis.

In receptor-mediated transcytosis, these larger molecules interact with specific receptors present on the luminal (blood-facing) surface of the endothelial cells that form the BBB. This interaction leads to the formation of a receptor-ligand complex, where the ligand represents the larger molecule (e.g., insulin or transferrin), and the receptor is located on the endothelial cell membrane.

The receptor-ligand complex is then internalized into vesicles within the endothelial cell. These vesicles transport the complex across the endothelial cell to the abluminal (brain-facing) surface. Once at the abluminal surface, the vesicles fuse with the endothelial cell membrane, allowing the larger molecules (insulin, transferrin, etc.) to be released into the brain's extracellular fluid.

Receptor-mediated transcytosis is a crucial mechanism that enables specific large molecules, essential for brain function and regulation, to cross the BBB and enter the brain. Understanding and utilizing this process is of great interest in the development of targeted drug-delivery strategies for treating neurological disorders and delivering therapeutic agents to the brain effectively.

B. Adsorptive-Mediated Transcytosis: Positively charged nanoparticles or peptides can bind to the negatively charged surface of BBB endothelial cells and undergo adsorptive-mediated transcytosis to cross the BBB (Haranath, Jonnala, et al., 2022; Sai et al., 2023; Vieira & Gamarra, 2016).

C. Other endocytic pathways

a) **Adsorptive Transcytosis:** Cationic (positively charged) molecules can cross the BBB via adsorptive transcytosis. The negatively charged endothelial cell membrane attracts cationic substances, allowing them to pass through the BBB.

b) **Transcellular Pathways:** Some molecules can penetrate the BBB through transcellular pathways, where they cross both the luminal and abluminal membranes of BBB endothelial cells. The exact mechanisms of transcellular transport for certain substances are still under investigation.

c) **Carrier-Mediated Transport:** Carrier-mediated transport involves the use of specific carrier proteins on BBB endothelial cells to transport certain substances across the barrier. These carrier proteins may facilitate the movement of drugs or molecules that share structural similarities with endogenous substances that are normally transported by these carriers.

4. *In vitro* and *In vivo* Studies:

A critical analysis of preclinical studies involving Trojan horse liposomes is presented in this section. It highlights the results of *in vitro* and *in vivo* experiments, including animal models that demonstrate the efficiency and selectivity of these liposomes in delivering drugs to the brain.

EVALUATION OF TROJAN HORSE LIPOSOMES

In vitro studies

These involve conducting experiments in a controlled laboratory environment to evaluate their characteristics, performance, and interactions with target cells or tissues relevant to BBB and brain delivery. These studies are essential for understanding the liposomes' behavior before progressing to *in vivo* (animal) and, eventually, clinical studies. Here are some common in vitro experiments conducted with Trojan horse liposomes (Ahad et al., 2020; Hathout et al., 2019; Li et al., 2020; Pardridge, 2010; Yan et al., 2022):

Liposome Characterization: *In vitro,* studies begin with the characterization of the liposomes, including their size distribution, zeta potential (surface charge), stability, and drug encapsulation efficiency. Techniques such as dynamic light scattering (DLS), TEM, and high-performance liquid chromatography (HPLC) are used for these assessments.

BBB Cell Culture Models: In vitro BBB models are essential tools for studying BBB penetration. BBB models can be established using primary brain endothelial cells or immortalized cell lines cultured on a permeable membrane. These models mimic the BBB's structure and function and provide a platform to study liposome transport across the barrier.

BBB Penetration Studies: BBB models are used to assess the penetration and transport of Trojan horse liposomes. Fluorescently labeled liposomes are often used to track their movement. The liposomes' ability to cross the BBB model is evaluated by measuring the amount of liposomal cargo that accumulates on the brain side of the barrier.

Cytotoxicity Assays: In vitro studies also assess the cytotoxicity of Trojan horse liposomes and their components on BBB endothelial cells. Cell viability assays, such as MTT or Alamar Blue assays, are commonly used to determine cell viability and assess potential adverse effects.

Targeting Ligand Validation: If targeting ligands are used on the liposome surface, their specificity and binding affinity to BBB receptors are assessed in vitro. Binding studies using fluorescently labeled ligands and receptor blocking experiments can validate their targeting efficacy.

Drug Release Studies: In vitro drug release studies are performed to understand the release kinetics of therapeutic agents from the liposomes under simulated physiological conditions. These studies help optimize drug loading and release profiles.

Intracellular Trafficking Studies: For liposomes that undergo intracellular uptake and trafficking, in vitro studies can examine their internalization pathways, such as receptor-mediated endocytosis or adsorptive-mediated transcytosis, using fluorescently labeled liposomes and confocal microscopy.

Stability Studies: In vitro stability studies assess the long-term stability of Trojan horse liposomes under various storage conditions, including temperature, pH, and light exposure.

In vivo studies

In vivo, studies of Trojan horse liposomes involve conducting experiments in live animals to evaluate their behavior, efficacy, and safety as potential drug delivery systems for crossing the blood-brain barrier (BBB) and delivering therapeutic agents to the brain. These studies are critical for assessing the liposomes' performance in a more complex and physiological setting, providing valuable data to guide their potential translation to clinical applications. Here are some

common in vivo studies conducted with Trojan horse liposomes (Lee et al., 2020; Li et al., 2020; Xia et al., 2008; Yan et al., 2022):

Animal Model Selection: Appropriate animal models, such as rodents (e.g., mice or rats), are chosen to mimic human physiology and BBB characteristics. Transgenic or disease-specific animal models may be used to investigate BBB alterations associated with specific neurological conditions.

Pharmacokinetic Studies: In vivo pharmacokinetic studies evaluate the liposomes' behavior in the body, including their distribution, circulation half-life, and clearance. These studies help determine how long the liposomes remain in the bloodstream and whether they accumulate in the brain.

BBB Penetration Studies: The main focus of in vivo studies is to assess the ability of Trojan horse liposomes to penetrate the BBB and deliver therapeutic agents to the brain. Fluorescently labeled liposomes or liposomes loaded with a contrast agent or drug are administered to animals, and their accumulation in brain tissue is quantified using imaging techniques such as fluorescence imaging, positron emission tomography (PET), or magnetic resonance imaging (MRI).

Targeting Efficacy: If targeting ligands are used on the liposome surface, in vivo studies evaluate their efficacy in targeting specific receptors on the BBB endothelial cells. This involves comparing the brain accumulation of targeted liposomes to non-targeted controls.

Therapeutic Efficacy: *In vivo* studies also assess the therapeutic efficacy of Trojan horse liposomes loaded with drugs. Animals with relevant neurological conditions or disease models are treated with liposomal formulations, and their therapeutic outcomes, such as tumor regression or disease improvement, are evaluated.

Biodistribution and Tissue Toxicity: Biodistribution studies investigate the distribution of liposomes in various organs and tissues beyond the brain. These studies help assess potential off-target effects. Tissue toxicity studies are performed to evaluate any adverse effects on non-target tissues.

Immunogenicity and Safety: *In vivo* studies evaluate the immunogenicity of Trojan horse liposomes, assessing potential immune responses triggered by liposome administration. Safety evaluations include monitoring for signs of toxicity or adverse reactions.

Brain Penetration Enhancement Strategies: In vivo, studies may also involve testing various strategies to enhance liposomal BBB penetration, such as modifying liposome surface properties or combining them with other BBB-crossing techniques.

THERAPEUTIC APPLICATIONS TROJAN HORSE LIPOSOMES

Absolutely, THL holds great promise in advancing treatments for a wide range of neurological conditions and disorders. Some of the potential applications include(Ahad, Haranath, Vikas, et al., 2021; Chinthaginjala et al.; Collet et al., 2013; Eltahir et al., 2023; Pardridge, 2020; Xia et al., 2007):

Neurodegenerative Diseases: These liposomes can deliver disease-modifying drugs, gene therapies, or neuroprotective agents to regions affected by neurodegenerative diseases like Alzheimer's, Parkinson's, and Huntington's disease, potentially slowing or halting disease progression.

Brain Tumors: Trojan horse liposomes can be employed to deliver chemotherapeutic agents or targeted therapies to brain tumors, improving drug delivery to the tumor site while minimizing effects on healthy brain tissue.

Central Nervous System Infections: Liposomes can deliver antibiotics or antiviral agents to treat infections such as meningitis or encephalitis within the central nervous system.
Gene Therapy: Engineered liposomes can carry therapeutic genes to address genetic neurological disorders, enabling the delivery of gene therapies across the BBB to correct genetic defects.
Stroke Treatment: Liposomes can deliver neuroprotective agents or drugs promoting brain tissue repair and recovery after a stroke, targeted to affected brain regions.
Pain Management: Liposomes loaded with pain-relieving drugs can be targeted to specific brain areas involved in pain perception, improving pain management while reducing systemic side effects.
Neuroinflammation and Immune Disorders: Trojan horse liposomes can deliver anti-inflammatory agents or immunomodulatory drugs to regulate immune responses within the brain, potentially benefiting conditions involving neuroinflammation or autoimmune neurological disorders.

CHALLENGES AND LIMITATIONS

The crucial limitations and challenges associated with THL are summarized as follows(Hathout et al., 2019; L. Jiang et al., 2016; Shi et al., 2014).
The complexity of the BBB: The BBB's intricate structure with tight junctions, efflux transporters, and limited endocytic pathways poses significant barriers to the efficient penetration of Trojan horse liposomes.
Heterogeneity of the BBB: Variability in BBB permeability across different brain regions and individual capillaries makes achieving consistent and targeted drug delivery challenging.
Choice of Targeting Ligands: Selecting appropriate targeting ligands with high specificity for BBB receptors while being compatible with the liposome surface can be difficult and may vary depending on the target and disease.
Intracellular Barriers: Even if liposomes cross the BBB, they must overcome intracellular barriers within brain endothelial cells for efficient transcytosis and drug release.
Cargo Release at Target Site: Ensuring the precise release of therapeutic agents carried by liposomes at the intended target site within the brain is critical for therapeutic efficacy.
Immunogenicity and Biocompatibility: The presence of foreign materials like liposomes in the body can trigger immune responses, potentially leading to clearance from the bloodstream or undesirable side effects.
Off-Target Effects: Delivery of drugs to the brain using liposomes may cause unintended off-target effects in non-brain tissues, leading to potential toxicities.
Scale-up and Manufacturing: Large-scale manufacturing of Trojan horse liposomes for clinical applications can be complex and costly, requiring consistent batch-to-batch production for regulatory approval.
Safety Concerns: Long-term safety of Trojan horse liposomes in chronic treatments needs comprehensive evaluation to address potential accumulation or toxicity concerns.

FUTURE PERSPECTIVES

The future prospects of THL for brain drug delivery hold great promise, and several areas of improvement and innovation are being explored (Ahad, Haranath, Pradeepkumar, et al., 2021; Jain, 2012; Pardridge, 2023; Roy et al., 2023):
Improved Targeting Ligands: Advances in molecular biology and biomarker identification may lead to the discovery of more effective and specific targeting ligands. This could enhance the

liposomes' ability to selectively cross the BBB and deliver therapeutic agents to precise brain regions.

Nanotechnology Advancements: Continued progress in nanotechnology and liposome engineering may result in more sophisticated formulations with improved stability, release kinetics, and BBB penetration capabilities. Smart and stimuli-responsive liposomes could be designed to release drugs in response to specific cues within the brain microenvironment.

Combination Strategies: Combining Trojan horse liposomes with other drug delivery approaches, such as focused ultrasound, nanoscale carriers, or peptide vectors, may synergistically enhance BBB penetration. These combinations could overcome challenges associated with individual approaches and lead to more efficient and targeted drug delivery.

Personalized Medicine: Advancements in precision medicine and genomics may enable the design of patient-specific Trojan horse liposomes tailored to individual genetic variations and BBB characteristics. Personalized liposomal formulations could improve drug delivery outcomes and treatment responses.

Theranostic Liposomes: The development of theranostic liposomes that can deliver therapeutic agents and provide diagnostic information could revolutionize neuroimaging and treatment monitoring. These liposomes could combine drug delivery capabilities with imaging agents for real-time monitoring of drug distribution and therapeutic response in the brain.

In Vitro BBB Models: Advances *in vitro* BBB models, including microfluidic devices and organ-on-a-chip technologies, could provide more accurate and predictive platforms for screening and optimizing Trojan horse liposomes before advancing to in vivo studies.

Translation to Clinical Trials: As preclinical studies continue to demonstrate the potential of Trojan horse liposomes, efforts to translate these findings into clinical trials are likely to intensify. Successful clinical trials could lead to the approval and commercialization of BBB-penetrating liposomal therapies for various neurological disorders.

Multi-Drug Delivery: Trojan horse liposomes could be designed to carry multiple therapeutic agents, such as a combination of chemotherapy drugs or gene therapies, offering more effective treatments for complex diseases or conditions with multiple underlying factors.

Regulatory Considerations: The development of Trojan horse liposomes for clinical use will require careful consideration of regulatory requirements and safety assessments. Collaboration between researchers, pharmaceutical companies, and regulatory agencies will be crucial for navigating the approval process.

CONCLUSION

This review highlights Trojan horse liposomes as a promising and innovative approach for brain drug delivery. With their ability to traverse the blood-brain barrier and target specific brain regions, these liposomes hold significant potential for treating neurological disorders and brain-related conditions. Despite existing challenges, ongoing research in improved targeting ligands, nanotechnology advancements, combination strategies, and personalized medicine offers promising solutions to enhance their therapeutic efficacy. The development of theranostic liposomes and advanced in vitro BBB models further support their clinical translation. Overall, this review contributes to the growing interest and investigation of Trojan horse liposomes as a game-changing strategy in the field of drug delivery to the brain.

ACKNOWLEDGMENTS

The authors are thankful to the college management for their encouragement and support.

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