Acupuncture and Herbal Medicine

Considerations regarding the application of Chinese herbal medicine-derived extracellular vesicle-like particles in a drug delivery system

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"Chinese herbal medicine extracellular vesicles-like particles" (CHM-EVLPs) refer to vesicle-like nanoparticles secreted by Chinese herbal medicines. These particles possess a lipid bilayer framework and contain various proteins, nucleic acids, and other active substances. Accumulating evidence suggests that CHM-EVLPs play crucial roles in regulating communication between mammals and plants, tissue repair, self-defense, and disease treatment. Their unique vesicle-like structure and excellent biocompatibility make them ideal drug delivery carriers for DNA and small molecule drugs. In recent years, studies focusing on utilizing CHM-EVLPs as delivery carriers have emerged as a prominent field in advancing the modernization of traditional Chinese medicine. However, several issues pertaining to security, standards in definition, methods of extraction and separation, and clinical application persist. The future research of CHM-EVLPs in disease treatment and drug delivery, along with potential challenges, is discussed in the following four sections.

Biogenesis, nomenclature, physical, and biochemical characteristics of CHM-EVLPs

Extracellular vesicle-like particles derived from plants were initially discovered by Halperin and Jensen[1] in the 1960s, who observed exosome-like particles secreted by carrot cells through transmission electron microscopy and defined them as multivesicular bodies (MVBs). Subsequently, with the identification of other plant-derived extracellular vesicles[2,3], numerous extracellular vesicle-like particles have been successfully isolated from Chinese herbal medicine sources, such as ginseng, ginger, turmeric, and aloe[4–7]. While a relatively mature theoretical framework exists regarding the biogenesis mechanism of mammalian extracellular vesicles, studies on the biogenesis mechanisms of CHM-EVLPs have not delved deeply enough. The major mechanisms have been categorized into the following three models: (i) MVB pathway; (ii) exocyst-positive organelle (EXPO) pathway; and (iii) vacuolar pathway. Despite these summarized secretion models, the specific secretion mechanism of CHM-EVLPs remains uncertain and necessitates further investigation[8].

Although plentiful CHM-EVLPs have been discovered, their nomenclature is varied and lacks unified standardization. The main reason for this is that the names of these particles consist of a Chinese herbal medicines part and a vesicle part. Firstly, the names of Chinese herbal medicines are different in many languages, including Latin, English, and Chinese pinyin. Secondly, the names of the vesicle parts also vary for different researchers. For example, "extracellular vesicles" "exosome-like nanoparticles" "nanovesicles" and "decoction bodies" all denote the same structures. Hence, the difficulty in establishing a standardized nomenclature. To promote the development and utilization of Chinese herbal medicine vesicle-like particles, the National Expert Committee on Research and Application of Herbal Vesicles has suggested unifying the various terms under the umbrella of CHM-EVLPs, aiming for acceptance among experts in the field of TCM. This authoritative organization might also delineate the specific scope of CHM-EVLPs, encompassing extracellular vesicles and extracellular vesicle-like particles exclusively derived from plant extracellular fluid and plant juices in plant-based Chinese herbal medicines.

The physical and biochemical characteristics of CHM-EVLPs closely resemble those of mammalian-derived extracellular vesicles. These properties include spherical morphology, nanoscale particle size, and Zeta potential ranging from neutral to –50 mV[9], as well as a similar major composition of lipids, nucleic acids, proteins, and metabolites[10]. Lipids serve as essential components for

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the membrane structure of CHM-EVLPs. The primary proteins found in CHM-EVLPs encompass membrane proteins, transmembrane proteins, and intracellular proteins. RNA represents the primary nucleic acid in CHM-EVLPs, while polysaccharides constitute the predominant metabolite compositions[11]. These critical components of CHM-EVLPs play an indispensable role in elucidating specific mechanisms related to human health, disease progression, and drug delivery. However, research on these main components has not been exhaustive, and further exploration is necessary to comprehensively understand their mechanisms.

Application of CHM-EVLPs in diseases

Due to the natural sources and high active concentrations of Chinese herbal medicines, CHM-EVLPs are considered excellent therapeutic agents with significant advantages[8]. Firstly, some CHM-EVLPs secreted by Chinese herbal medicines are homologous to food and certified non-toxic[4–6]. Secondly, the absence of human disease-causing pathogens in plants expands the potential applications of CHM-EVLPs[8]. Thirdly, in contrast to extracellular vesicles derived from mammals, CHM-EVLPs possess the advantages of being sustainable, cost-effective, and easily acquired. Therefore, CHM-EVLPs have been widely used for skin regeneration, anti-inflammation, immune regulation, anti-tumor treatments, the repair of alcoholic liver injuries, and other diseases[8–10].

In a study conducted by Liu et al.[12], turmeric EVLPs were prepared from the rhizome of turmeric and demonstrated excellent anti-inflammatory and antioxidant effects, primarily through the regulation of the NF-κB signaling pathway. Zhang et al. obtained ginger EVLPs using centrifugation and sucrose gradient, which showed inhibition of colitis in mice by activating the aryl hydrocarbon receptor (AHR) pathway and inducing interleukin-22 (IL-22) expression[13]. Cao et al.[9] discovered that ginseng EVLPs had an anti-melanoma effect by altering macrophage polarization and reprogramming the immunosuppressive tumor microenvironment. Another study suggested that the application of ginseng enhanced the efficacy of immune checkpoint antibodies by modulating the immunosuppressive microenvironment[14]. Additionally, CHM-EVLPs have potential value in tissue repair, cell growth promotion, and immune response regulation, as they travel along capillaries to target injury sites and promote the elimination of inflammation and healing of wounds.

Application of CHM-EVLPs in the delivery system

The application of CHM-EVLPs in the delivery system is a crucial aspect of clinical drug development and application. Various studies have demonstrated the potential of animal-derived extracellular vesicles to deliver chemotherapeutics, photosensitizers, therapeutic antibodies, and small interfering RNA (siRNA)[6,8]. However, the clinical translation of mammalian-derived extracellular vesicles into suitable delivery carriers is hindered by high manufacturing costs, complex separation and purification processes, and immunogenicity[8,15,16]. In contrast, CHM-EVLPs offer advantages such as widespread availability, higher circulation stability, safety, low toxicity, high rigidity, and low immunogenicity compared to mammalian-derived extracellular vesicles and artificial carriers. Recent studies have successfully utilized CHM-EVLPs-based nanoscale carriers to deliver both hydrophobic and hydrophilic medicines[8,15].

The protein-lipid membrane structure and specific vesicle inter-coelom of CHM-EVLPs can effectively facilitate the loading of active ingredients under suitable conditions, including co-incubation, sonication, electroporation, and freeze-thaw processes. The current application of CHM-EVLPs in drug delivery primarily involves two approaches. Firstly, isolated CHM-EVLPs are used to encapsulate drugs without modification. Secondly, CHM-EVLPs are processed and recombined to generate engineered vesicles[8]. The current research aims to utilize CHM-EVLPs as carriers for several purposes: (i) prolonging blood circulation, (ii) improving tissue penetration to reach the target site, (iii) differentiating between tissues and organs, (iv) maintaining effective release of therapeutic agents from carriers, (v) enhancing encapsulation efficiency, (vi) reducing toxicity and drug resistance, and (vii) improving biocompatibility[8,15–18].

CHM-EVLPs as the drug carrier without any modification

Most studies have demonstrated that using CHM-EVLPs directly as drug carriers offers advantages such as stability, negative surface zeta potential that effectively prolongs drug circulation time, and simple preparation methods[18,19]. Active ingredient agents can be directly encapsulated into CHM-EVLPs through appropriate methods such as co-incubation, sonication, and electroporation. Wang et al.[19] successfully loaded the anti-STAT3 inhibitor JSI-124 into grapefruit-derived vesicles, which efficiently accumulated in solid tumors and exhibited inhibitory effects on glioma growth. Zhang et al.[20] utilized ginger EVLPs to load siRNA-CD98, demonstrating effective targeting of colon tissue. In another study, ginger EVLPs were employed to load the therapeutic agent doxorubicin, showing superior drug release properties compared to the commercial liposomes used as delivery carriers[21].

Engineering CHM-EVLPs as the drug carrier

In addition to their natural origin, safety, low toxicity, and low immunogenicity, CHM-EVLPs are highly scalable for large-scale production, making them ideal candidates as sustainable drug carriers[8]. With the development of functional delivery carriers, many researchers have engineered CHM-EVLPs to achieve multiple delivery targets[18,19].

The common methods of preparing engineered CHM-EVLPs involve isolating lipids from CHM-EVLPs and recombining them into semi-artificial cargo carriers or embedding them in protein receptors. Compared to artificial liposomes, engineered CHM-EVLPs are associated with low cost, low immunogenicity, uniform particle size, specific targeting ability, and a wider range of applications[19]. In a study conducted by Lu et al.[15], celery-EVLPs were selected as delivery carriers to

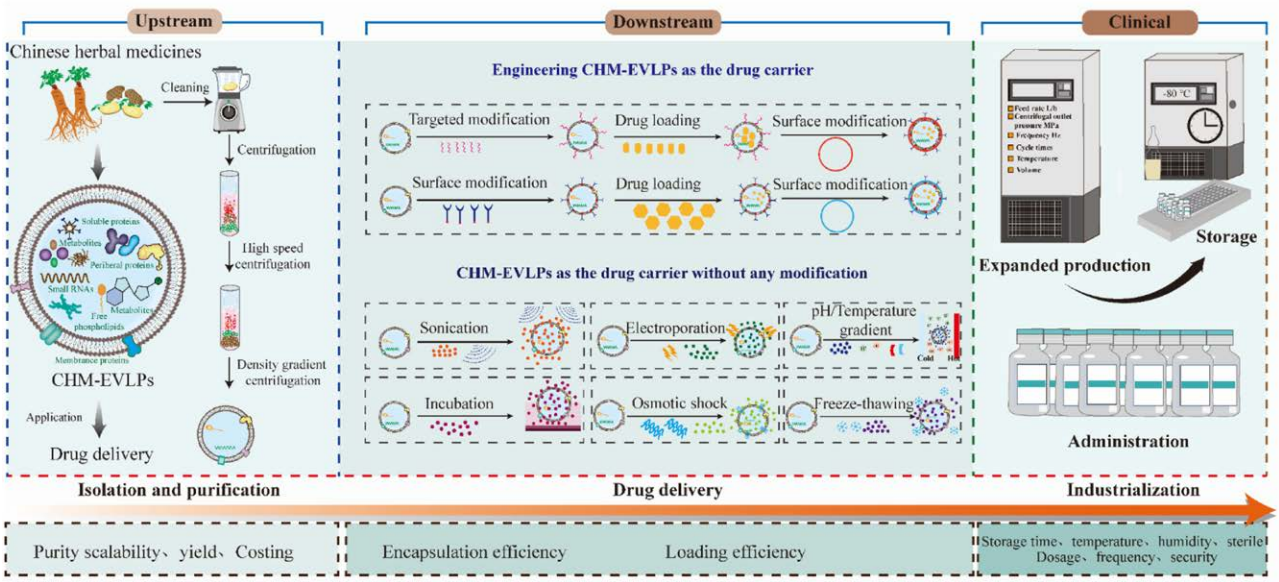

Figure 1. The development and critical problems of CHM-EVLPs-based nano-drug delivery systems can be divided into upstream and downstream processing and preclinical testing. CHM-EVLPs: Chinese herbal medicine extracellular vesicles-like particles.

encapsulate doxorubicin and engineered to be composite carriers. Compared to conventional synthetic carriers, the composite carrier demonstrated superior efficacy in inhibiting tumor growth[15]. Similarly, a study conducted by Li et al.[5] utilized ginger EVLPs as the delivery carrier, modified with folic acid on the surface to enable specific targeting. This modification facilitated effective accumulation in carcinoma cells and demonstrated remarkable improvement in tumor inhibition efficacy[5].

Although CHM-EVLPs can serve as singular, engineered delivery carriers due to their excellent characteristics, several potential issues should be considered and analyzed (Figure 1). Firstly, the potential interaction between CHM-EVLPs and encapsulated agents needs to be further explored and investigated. CHM-EVLPs contain a wide range of phytochemicals, which may induce adverse effects and lead to drug-carrier interactions when used as drug-delivery carriers. For example, Kiani and Imam discovered that the flavonoids present in grapefruit-derived extracellular vesicle-like particles could inhibit drug metabolism and result in toxic reactions[22]. Secondly, there is a lack of a standardized guidance system to regulate the application of CHM-EVLPs as delivery carriers. Thirdly, when CHM-EVLPs are utilized to deliver active substances of Chinese medicine, compatibility needs to be studied in-depth. Fourthly, the varying delivery efficiency of CHM-EVLPs derived from different production areas requires further exploration. Fifthly, as Chinese medicinal liquid is highly unstable, storage issues need to be addressed when CHM-EVLPs are produced on a large scale.

Conclusion

Despite widespread reports of the therapeutic and delivery effects of CHM-EVLPs, research into the biogenesis, release, and functioning mechanisms of CHM-EVLPs remains limited. Standardization requires the elucidation of multiple isolation methods and the characteristic features of CHM-EVLPs. Additionally, the identification of characteristic markers of CHM-EVLPs is necessary to optimize the isolation and purification process. Concerns regarding safety and drug interactions also require thorough investigation.

Therefore, based on the current research status of CHM-EVLPs as drug carriers, the following recommendations are presented. First, a comprehensive database of CHM-EVLPs must be established to provide reliable data for selecting properties exhibited by CHM-EVLPs as drug delivery carriers. Second, the intrinsic connection between therapeutic and cargo-delivery applications of CHM-EVLPs needs further elucidation.

Conflict of interest statement

The authors declare no conflict of interest.

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Author contributions

Ruoning Wang and Liuqing Di conceived and designed this commentary. Fucai Chen and Yingjie Zhang drew the picture and summarized the results. Yu Zheng, Longxiang Pan, and Jiale Li reviewed and edited this commentary. Peng Cao, Ruoning Wang, and Liuqing Di revised this commentary. All of the authors have read and approved the final manuscript.

Ethical approval of studies and informed consent

Not applicable.

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Data availability

All data generated or analyzed during this study are included in this published article.

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