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Lyotropic Liquid Crystal Precursor as an Innovative Herpes Simplex Virus Vector for Melanoma Therapy †

Fangqin Fu [1,2,‡], Wenhao Wang [1,‡], Yukun Gu [1,3], Zhengwei Huang [4,*], Ying Huang [4,*], Xin Pan [1] and Chuanbin Wu [1,4]

[1] School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou 510006, China; email1@gmail.com (F.F.); email2@gmail.com (W.W.); email3@gmail.com (Y.G.); email4@gmail.com (C.W.)
[2] School of Medical and Pharmacy, Ocean University of China, Qingdao 266003, China
[3] Shanghai Ghost Consulting Co., Ltd., Shanghai 200241, China
[4] College of Pharmacy, Jinan University, Guangzhou 510632, China
* Correspondence: hzhengw3@163.com (Z.H.); huangy2007@jnu.edu.cn (Y.H.)
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‡ These authors contributed equally to this work.

Abstract: To overcome the low efficiency, toxic side effects and high recurrence of traditional therapy for malignant melanoma, an in-situ gel system HSV-LLCP was developed as a local treatment for malignant melanoma in this study. This system was based on lyotropic liquid crystal precursor (LLCP) loading with oncolytic virus herpes simplex virus-1 (HSV-1). With the unique lattice structure, HSV-LLCP could enhance the stability of HSV-1 and arrest HSV-1 at the injection site. The performance of LLCP as a virus vector was evaluated comprehensively. The HSV-LLCPshowed a rapid gelling property (within 2 s) and the shear viscosity ranged from 5 to 9 mPa·s. The result also revealed the outstanding stability of HSV-LLCP. The release behavior showed a triphasic sustained-release pattern during the experiment period. In addition, HSV-LLCP exhibited a superior oncolytic activity compared to HSV-1 solution in murine melanoma B16 cells. This study showed that HSV-LLCP would become an alternative and promising HSV-1 vector with high safety and stability for melanoma treatment in the clinic.

Keywords: lyotropic liquid crystal precursor; herpes simplex virus; melanoma; virus vector

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1. Introduction

Melanoma is a life-threatening skin cancer, imposing significant burdens to globe healthcare systems [1]. The primary treatment for melanoma is surgical resection supplemented by chemotherapy [2]. However, surgical resection could lead to unbearable pains in patients and the risk of recurrence cancers if incomplete resection of the tumor tissue occurred. In addition, chemotherapy could lead to unexpected toxic side effects and tumor resistance [3]. To date, nonsurgical treatments have been increasingly employed for melanoma therapy, including nonspecific immune adjuvants, cancer-specific vaccines, monoclonal antibodies and specific immunostimulants. Oncolytic virotherapy has become the frontier of biological therapy for tumor treatment in recent years. With The U.S. Food and Drug Administration (FDA) approval of the only oncolytic immunotherapy approach Imlydic (talimogene laherparepvec, T-VEC) for the treatment of melanoma, a genetically modified herpes simplex type 1 virus, oncolytic virotherapy using Herpes Simplex Virus-1 (HSV-1) has been successfully applied for the treatment of melanoma [4–6].

The main reasons include: (1) HSV-1 can exert increased antitumor activity via triggering a tumor-specific cytotoxicity T cells responses [7]; (2) gene editing of HSV-1 by some mutants may reduce the invasiveness towards nontargeted systems [8]; (3) undesired viral replication of HSV-1 can be effectively controlled by antiherpetic agents such as acyclovir [9].

However, current commercial formulations using HSV-1, such as Imlydic requires strict storage conditions (−80 °C) to cope with its unstable characteristics. In addition, direct injection of HSV-1 may lead to virus migration to nontumor tissues or cause unexpected immune response representing a therapeutic risk, such as chronic granulomatous dermatitis [10]. For these concerns, we developed a HSV-loaded lyotropic liquid crystal precursor (LLCP) system with in situ gelation properties as the vector for HSV-1, improving storage conditions (−4 °C) and enhancing the storage stability via protecting HSV-1 with its unique crystal lattice [11]. Upon contacting with water at the injection spot, LLCP transforms into the solid-state lyotropic liquid crystal gel (LLCG), and then locked by crystal lattice which can prevent the migration of HSV-1 to nontumor sites [12] and prohibit their rapid clearance from blood circulation owning to their blinding to plasma protein and the host system defense involving mononuclear macrophage system[13]

In this study, an in-situ gel system HSV-LLCP was developed. The preparation design is shown in Figure 1. Preparation characterizations and in vitro efficacy analyses were performed to evaluate the feasibility of LLCP as HSV-1 vector. The results show that this system has good stability and low leakage tendency, and is a promising candidate for the treatment of melanoma.

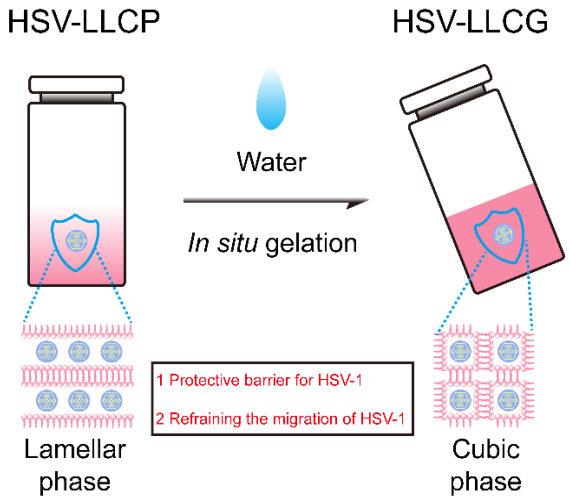

Figure 1. Schematic illustration of the preparations design in this work.

2. Materials and Methods

HSV-LLCP was fabricated by HSV-1, inositol and sorbitol containing phosphate buffer solutions (PBS), glyceryl monooleate and Pluronic F127, according to the proprietary procedure (CN201611247953.3). The HSV-1 loaded LLCP (HSV-LLCP), HSV-1 loaded LLCG (HSV-LLCG) and HSV-1 containing solution (HSV-Sol) were used in subsequent experiments.

Characterizations of preparation: The gelation time of HSV-LLCP in PBS at 37°C was recorded by a timer. The phase behaviors of HSV-LLCP and HSV-LLCG were examined using a polarized optical microscopy (POM, Micro-shot Technology Co., Ltd., Guangzhou, China) at ambient temperature. The shearing viscosity of HSV-LLCP was measured using a Kinexus Lab+ rotational rheometer (Malvern Instruments Ltd., Worcestershire, UK) from 10-1 to 102 s-1 at 37 °C. The stability of virus titer of HSV-LLCP, HSV-LLCG and HSV-Sol within 28-day storage at 4 °C was investigated using plaque assays.

In vitro efficacy profiles: The release profiles of HSV-1 from HSV-LLCG and HSV-Sol were examined in PBS media for 2880 min, and model fitting of the release curves was conducted. The replication of HSV-1 in B16 cells (2 × 105 cells/dish) was investigated using MTT assay at multiplicities of infection (MOI) from 0.003 to 0.3 PFU/cell [14]. MTT assay was also employed to test the cytotoxicity of HSV-LLCP, HSV-LLCG and HSV-Sol in B16 cells at an MOI level of 0.003 PFU/cell.

Statistical analysis: The statistical significance of the differences between test groups was analyzed by Student's *t*-test. When *p* value was less than 0.05, the difference was considered significant.

3. Results and Discussion

The gelation time of HSV-LLCP was 1.34 s. The rapid gelation in aqueous environment allows the transfer of HSV-LLCP into HSV-LLCG after injection, that can further serve as a depot for HSV-1. The POM images of HSV-LLCP and HSV-LLCG exhibited cruciate flower texture and dark site, suggesting their lamellar phase and cubic phase, respectively (Figure 2A,B) [15]. The shear viscosity ranged from 5 to 9 mPa·s (Figure 2C), which is suitable for an injection system [12]. All groups revealed good stability within seven days, while the virus titer of HSV-1 subsequently dropped significantly. On Day 14 and 28, the virus titer of HSV-LLCP and HSV-LLCG was significantly higher than that of HSV-Sol ($p < 0.05$). Figure 2D showed that the virus titer of HSV-LLCP was lower than that of HSV-LLCG ($p < 0.05$), indicating that the gel state in cubic phase can provide stronger protective effects. HSV-LLCP could be stored at 4 °C, which can be easily achieved. These characteristics of HSV-LLCP facilitates the application as a stable and convenient vector for oncolytic virus delivery.

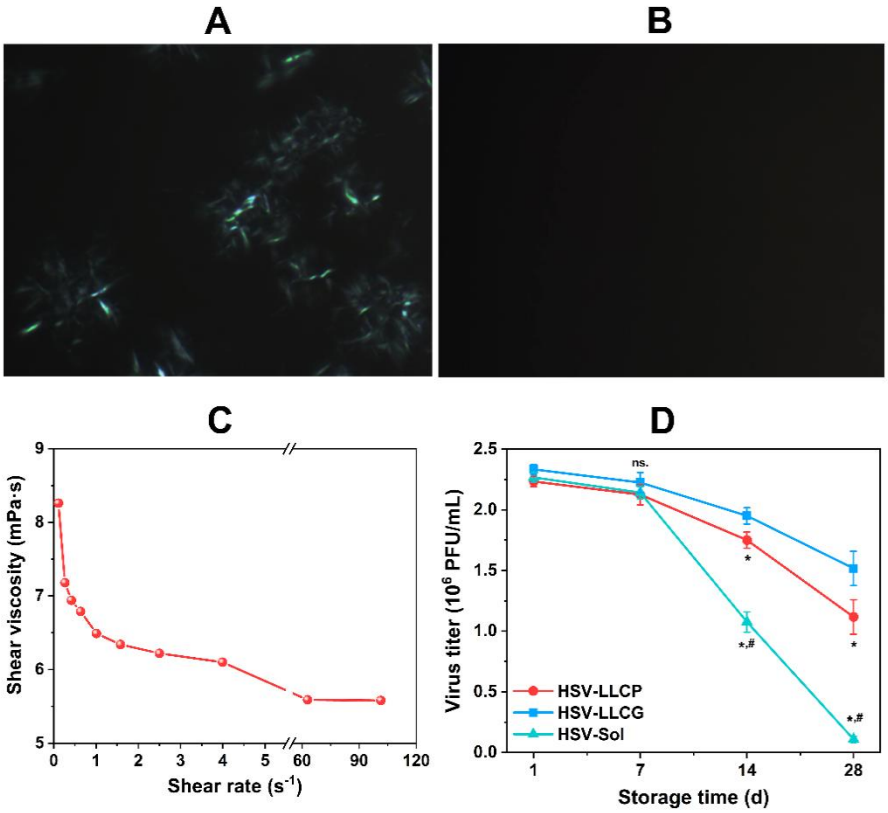

Figure 2. Preparations characterizations. (**A**) POM image of HSV-LLCP; (**B**) POM image of HSV-LLCG; (**C**) Shear viscosity of HSV-LLCP; (**D**) Stability of virus titer of HSV-LLCP, HSV-LLCG and HSV-Sol (n = 3). ns.: not significantly lower than day 1 ($p > 0.05$); *: significantly lower than HSV-LLCG at the same day ($p < 0.05$); #: significantly lower than HSV-LLCP at the same day ($p < 0.05$).

Upon encountering water, HSV-LLCP spontaneously transformed into HSV-LLCG. Therefore, we evaluated in vitro efficacy of HSV-LLCG. The release profile of HSV-1 exhibited a triphasic sustained-release pattern (Figure 3A): (I) 0~90 min, rapid release; (II) 90~540 min, slow release; (III) 1440~2880 min, plateau phase. XXXX simulation using Origin 2018 suggested that Ritger-Peppas model was the best-fitted model with a correlation coefficient R2 = 0.9026. When the kinetic exponent n ≤ 0.43, the drug release mechanism was Fickian Diffusion depending on Ritger-Peppas model. When n = 0.85 and 0.45 < n < 0.85, a Case II transport and intermediate transport mechanisms dominated the release process, respectively. The obtained exponent n = 0.2961 < 0.43, implying that HSV-1 released in a Fickian diffusion manner (concentration-dependent) [16]. In contrast, the release of HSV-Sol was instant, which could cause unexpected side effects due to the high virus concentration, and even could transport to nontumor sites. The replication of HSV-1 in B16 cells was demonstrated by the viability of cells infected by different MOI levels (Figure 3B). With the increases of MOI level and incubation time, the viability of B16 cells substantially decreased, suggesting that the cytotoxicity of HSV-1 was induced by viral replication. Furthermore, the cytotoxicity of blank LLCG, HSV-LLCG and HSV-Sol was shown in Figure 3C. MTT assays showed that HSV-LLCG and HSV-Sol had higher cytotoxicity compared to blank LLCG ($p < 0.05$), indicating their strong oncolytic activity. HSV-Sol showed higher toxicity than HSV-LLCG within 48-h incubation ($p < 0.05$), whereas at 72 h, the cytotoxicity of them showed no significant difference ($p > 0.05$). This could be attributed to the sustained-release of HSV-1 from HSV-LLCG. Taken together, HSV-LLCG exerted a sustained-release profile and an acceptable oncolytic activity in vitro.

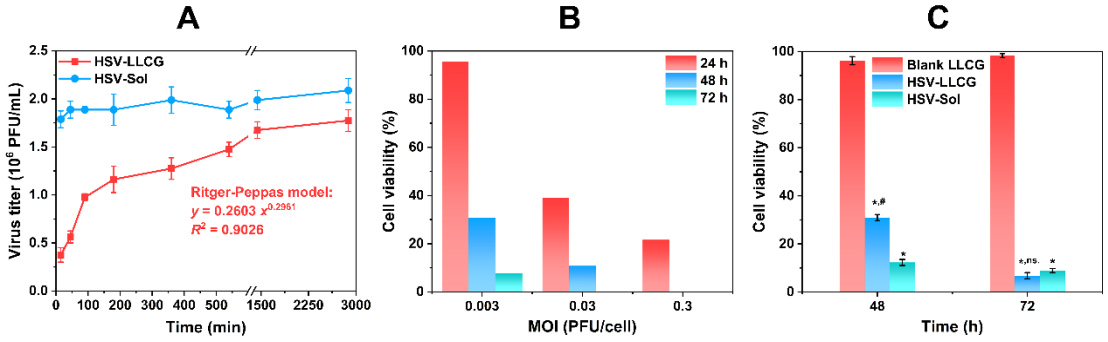

Figure 3. Results of in vitro efficacy evaluations. (**A**) Release studies of HSV-LLCG and HSV-Sol (n = 3); (**B**) In vitro replication of HSV-1 in B16 cells, expressed as the cell viability compared to uninfected cells (mean of three replicates); (**C**) Cytotoxicity of blank LLCG, HSV-LLCG and HSV-Sol in B16 cells (n = 3). *: significantly lower than blank LLCG at the same time ($p < 0.05$); #: significantly higher than HSV-Sol at the same time ($p < 0.05$); ns.: no significant difference with HSV-Sol at the same time ($p > 0.05$).

The unique lattice structure of cubic liquid crystals transformed from lamellar liquid crystals after encountering the aqueous condition could endow HSV-1 a stable and confined environment which isolated HSV-1 from the external environment and endued HSV-1 a sustained-release pattern (Figure 4). This confined environment shielded the clearance of HSV-1 from circulation and contributed to the realization of "viremic threshold," which was pivotal for the spread of therapeutic viruses [17].

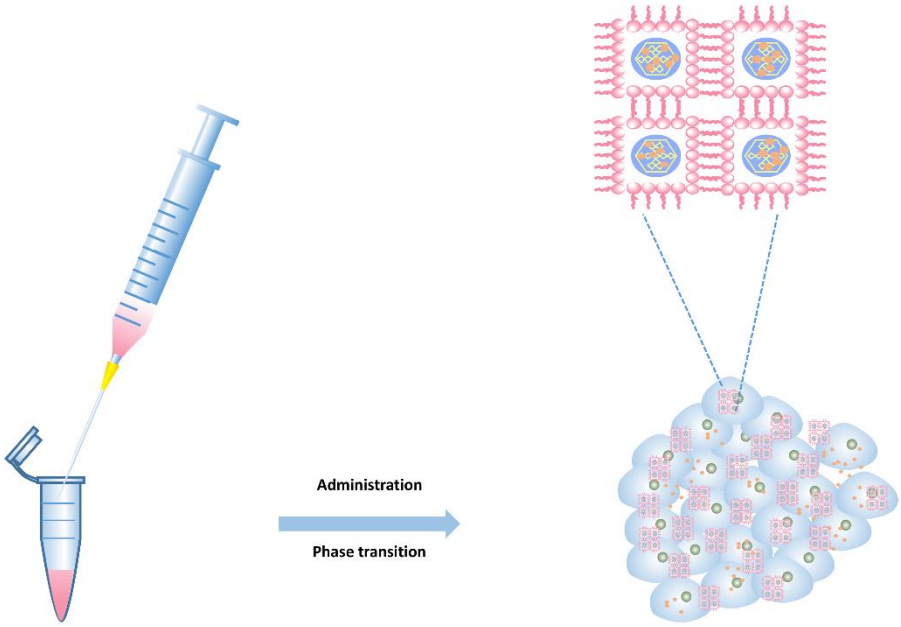

Figure 4. The diagrammatic sketch of HSV-LLCP system after administration.

4. Conclusions

In this study, a novel in-situ HSV-LLCP system was developed as a local treatment for malignant melanoma, with its feasibility as HSV-1 vector investigated. The transition from LLCP to LLCG prevented HSV-1 from inactivating and penetrating into surrounding tissues, providing high stability and low leakage tendency when applying HSV-1. Moreover, HSV-LLCP could be stored at 4 °C, an easier storage condition. The HSV-LLCG also exhibited a moderate in vitro cytotoxicity and replication in murine melanoma cells, and possessed a sustained-release profile. Our results demonstrate that HSV-LLCP system is a promising vector for oncolytic therapy and could be shifted to clinical use with great potential. Prior to further applications, the pharmacokinetics and antitumor mechanisms of HSV-LLCP will be investigated in our lab.

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