Precise Engineering of Nanoassembled Corilagin Small Molecule into Supramolecular Nanoparticles for the Treatment and Care against Cervical Carcinoma

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Abstract

Cervical cancer is one of the most frequently diagnosed tumors in the world and responsible for a significant percentage of women's deaths. In spite of advances in clinical and medical techniques, cervical cancer is indeed a big challenge. Latest treatments to breast cancer include surgical reduction, radiotherapy, estrogen treatment and the use of multiple chemotherapeutic medications. However, drug resistance, related severe adverse reactions, metastases and recurrence risks do need to be tackled, involving safe and alternate methods. Herein, we present tumor-specific targeting supramolecular nanoassembly, and therapeutically to overcome the different challenges raised by the distribution of the pharmaceutical potential anticancer drug Corilagin. On covalent conjugations of hydrophobic linoleic acid by carboxylic group, the Corilagin prodrugs were skilled in impulsively nanoassembly into extremely stable nanoparticles size (~ 100 nm). Electron microscopic techniques have examined the newly synthesized morphology of Corilagin-NPs. The anticancer properties of Corilagin and Corilagin-NPs against CaSki and HeLa (cervical carcinoma) cancer cell lines have been evaluated after successful synthesis. Other research, such as dual staining acridine orange/ethidium bromide, Hoechst 33344 and flow cytometry study on the apoptosis mechanisms, have shown that proliferation in cervical cancer cells is associated with apoptosis. Compared to Corilagin, Corilagin-NPs demonstrate excellent biocompatibility, this study clarified the Corilagin-NPs as a healthy and cervical cancer care chemotherapeutics technique and deserve further clinical evaluations.

1. Introduction

Cervical cancer, with its incidence gradually growing over the last couple of years, is one of the normal endocrine malignancy cells. Cervical malignancy is usually produced from the epithelial stem of cervical follicular cells, and can also be classified into well-distinguished cancer cells, less discriminated malignancy, and cancer activities made by anaplastic malignancy tumor cells [1–6]. While the common of cervical cancers have a favorable prognosis, cervical cancers have barely any successful therapeutic approaches. For patients with painful local invasions or distant metastasis, conventional treatment methods are typically not successful, and patients with distant metastasis also depreciate, with a five year survival rates of nearly 50% [7–10].

Corilagin and its deoxycytosine derivatives offer ideal chemotherapy for the pre-clinical treatment of numerous cancer cells. In cervical cancer metastasis, Corilagin integrates elongations of the DNA terminal chains to replicate, ultimately activating tumor cell apoptosis. Although these Corilagin analogues have been used to treat cervical patients, treatment outcomes appear to be extremely unsatisfactory, improving the median survival of cervical patients by just one month [11–16]. The Corilagin pre-clinical efficiency is seriously hampered by crucial hurdles like the half-lifer time blood flow rate, accelerated deactivation and defecation, the creation of drug tolerance, and the possible side effects. Therefore, there is an important impetus for the increase of the net of basic but well-organized methods to totally connect Corilagin, which will increase the effectiveness of this type of medication for cervical cancer demoralization management [17–21].

For the treatment of different diseases, drug delivery nanoassembly accomplished of controlling the physico-chemical behavior and efficiency of the in vivo pharmaceutical drugs given are evolving. Corilagin also has hydrophilic fragments and is thus discordant with various polymeric nanoparticles commonly used in the precise delivery of chemotherapeutic drugs. The significant difficulty is however defined by the biological processes for initiating composition with hydrophilic Corilagin moiety with polymeric system [22–26]. This highlights the difficulties and increases the viability of the nanoassembly and the biocompatibility of the management of Corilagin cervical cancer using various methods. Recently, with PEG₂₀₀₀ micelles, Corilagin and its prodrugs have been integrated into the admirable anticancer agents mentioned by Kwon and co-workers [27–30]. In recent times, injectable nanomedicines using Corilagin have been documented by Cattell and study groups, and their analogues display superior water solubility and biocompatibility. The in vitro proliferation ability of small molecules of prodrug identified with free Corilagin fragments was not affected by Corilagin amino group acylation [31–35].

In addition, unperformed modification of Corilagin to inactive metabolites correspondents may be significantly inhibited after systemic control due to reduced metabolic properties of in the plasma membranes. Therefore, the Corilagin encapsulation prodrug was shown to reduce drugs stimulations and increase the performance of drug loading efficacy by the effects of permeability and retention of enhancement [36–40]. In this report, we defined simple and superior method of effect that energetically combine with the supramolecular nanoassembly tumor targeting and drug reconstruction therapies of Corilagin. To effectively targets the objective, hydrophobic linoleic acid (LA) is an integral fatty acids, by carboxylic group formation, was conjugated with the amino moiety of the Corilagin equivalent. Fruitfully, we discovered in the water solutions the Corilagin prodrugs of increasing self-modeling into the nanoparticles of nanoassembly. The in vitro proliferation of cervical cancer cells was investigated by the nanoassembly Corilagin prodrug and the morphological deviations and cell death examinations of cervical cancer cells were identified.

2. Experimental Part

2.1. Fabrication of Corilagin-NPs

DIEA was combined with Corilagin (75 mg, 0.20 mol) and hydrophobic linoleic acid (78 mg, 0.20 mol) solutions containing 2 mL of DMF (103 mg, 8.1 mol). The reaction mixture solutions was allowable to stir overnight at 37 °C and then to evaporate to extract the DMF solution. The reaction mixtures that have been absorbed in the DCM. Five percent citric acid, NaCl and aqueous NaHCO₃ is used to wash the organic coating. The DCM solution were removed using vacuum dried with Na₂SO₄ solution. Figure 1 demonstrates the comprehensive fabrication techniques.

2.2. Characterization

The morphology of Corilagin-NPs was tested by transmission electron microscopy (TEM; JEM-1200EX, Japan) ensuing with phosphotungstic acid staining (2%, w/v) for 3 min, and Corilagin-NPs were then

perceived and examined by employing them on films of copper grid-irons. The polydispersity index (PDI), zeta potential and particle size of nanoparticles were examined using the Zetasizer ZEN3600 (Malvern, England).

2.3. Cell culture

CaSki and HeLa cervical cancer cell lines were cultured in DMEM (Gibco, NY, USA) enclosing 10% fetal calf serum (Hyclone, Logan, UT, USA), 100 µg/mL streptomycin (Life Technology, NY, USA), 100 IU/mL penicillin (Bioscience Technology, NY, USA), 1% L-glutamine (Bioscience Technology), and 1% non-essential amino acids (Bioscience Technology) at 37°C, 95% and 5% for humidity and CO₂ respectively.

2.4. In vitro proliferation assay by MTT

According to previously recorded protocols [41–44], a MTT assay was achieved. It is used to measure Corilagin and Corilagin-NPs for in vitro cytotoxicity. The cells of CaSki and HeLa were plated 2×10^4 cell per well in a 96-well culture plate for the attachment followed by cultured for 24 h at 37°C. 10 µL of the MTT solution was applied to each well after incubation at 37°C for 24 hours, and the 525 nm absorbance was analyzed 2 hrs later by Eliza microplate reader (TECAN Sunrise, Switzerland). The percentage of the cell proliferation were calculated by using the graph pad prism software. As seen below, the cell viability (percent) was evaluated using the formula;

$$\% \text{ of cell viability} = \text{OD}_{\text{treated}} / \text{OD}_{\text{control}} \times 100$$

2.5. Cellular Uptake

According to previously recorded protocols, a cellular uptake assay was performed. In 6-well plate at 1×10^4 CaSki and HeLa cervical cancer cells were pictured confocal laser scanning microscopy (LSM 510 Meta; Zeiss, Germany). Lysotracker Corilagin-NPs with Dil (Molecular Probe, USA) stained the cell lysosome (red color), then DAPI stained the nuclei. Images were examined by confocal laser scanning microscopy (CLSM) utilizing the image analyzer software manufacturer's protocol ^{45–48}.

2.6. In vitro drug release profiles

The controlled cumulative release of the Corilagin and Corilagin-NPs have been assessed using membrane dialysis (Spectrum Laboratory, 14 kDa-molecular weight). In short, 100 µg/mL of Corilagin-NPs (Corilagin concentration) is dialyzed at 37°C to 20 mL of phosphate buffer solutions containing Tween 80 (0.2%) at pH 7.4. The releasing medium was obtained by constant mild stirring 37°C in an orbital shaker incubator (100 RPM) at and an equivalent amount of supplemented fresh medium.

2.7. Acridine orange and ethidium bromide (AO/EB) and Hoechst 33344 staining

AO/EB and Hoechst 33344 staining fluorescent dyes which bound strongly to the chromatin and subsequently shine more fluorescent upon bounding. Apoptotic morphological improvements upon treating with IC₅₀ concentrations of Corilagin and Corilagin-NPs against CaSki and HeLa cancer cell lines

were assessed. After staining, the cells at 20 X magnification were displayed under a fluorescence microscopy (Olympus IX81, Germany) [49–52].

2.8. Flow cytometry techniques

Flow cytometry was completed to study modes of cell death over FITC-Annexin V and propidium iodide (PI) based apoptosis detection assay kit (Invitrogen, Thermo Fischer Scientific, USA) followed by previously reported protocol [53–56]. The CaSki and HeLa cells were through the trypsinization to avoid the cell death. Trypsinized the cells and twice washed with PBS. The cells supernatant was removed and the cell pellets was suspended in a 250 μ L 1 $\times$ binding buffer (from the kit) with 5 μ L of FITC Annexin-V and PI was added and incubated with 37°C in dark conditions. After 10 minutes, the samples were subjected to flow cytometry analysis using FACS Canto TM II, BD Biosciences.

2.9. Hemocompatibility assay

Human blood samples were attained from the Nursing Department, the Second Affiliated Hospital of Xi'an Medical College, Shaanxi Province, China. Red blood cells (RBCs) were collected by centrifuging the samples at 1800 rpm at 5°C for 5 min. RBCs is washed three times with PBS and suspended in 4 ml of PBS. Next, 0.1 mL of diluted RBCs is applied to the Corilagin and Corilagin-NPs in 0.5 ml PBS suspension at the corresponding concentrations and incubated for 4 h. After incubation, the samples were moved to 96-well plates. Hemolytic activity was calculated by calculation at an absorbance of 570 nm. Monitor samples of the buffer lyses and 100 percent buffer lyses were also analyzed in these experimental procedures. The proportion of hemolysis was determined as follows:

$$\% \text{ hemolysis} = (A_s - A_n) / (A_p - A_n) \times 100\%$$

Whereas denotes “the absorbance of Corilagin and Corilagin-NPs at various concentrations (5, 10, 15),” and A_n and A_p denote the negative and positive controls, respectively.

3. Results And Discussion

3.1. Fabrication and characterisation of Corilagin

Figure 1 demonstrates the complete synthetic procedure. Corilagin analogs conjugated with a number of successive groups at the carboxylic terminal ends demonstrate improved stability in cellular membrane due to the dehydrogenation purpose. In addition, LA hydrophilic regulator is used for the development of polymeric prodrugs. In this work, we planned and produced the Corilagin analogs for the LA polymer chains to render the Corilagin-LA prodrug (Fig. 1). Conjugation was achieved by coupling Corilagin to LA under the structure of Corilagin-LA, and the Corilagin-LA prodrugs were filtered using thin layer chromatography (TLC) and column chromatography approaches with the high yield (80.5 %). The lipophilic design of the drug delivery systems, which create nano-assemblies, could be synthesized in water by nanoassembly approaches without the addition of any surfactants. In addition, the organic phases of the Corilagin-LA prodrugs are absorbed in the solution of DMSO into the DD-H₂O aqueous

suspensions. More importantly, the elimination of excess organic solvents through double distilled water from the fabricated nanoparticles rather than prodrug accumulation. In addition, TEM observations were conducted to determine the structure of supramolecular nanoassembly Corilagin-NPs (Fig. 2A). The results of the supramolecular nanoassembly Corilagin-NPs showed that the well-structured form with a sphere-shaped was $\sim 80.3 \pm 3.12$ nm (Fig. 2B). In addition, the hydrodynamic parameter of the nanoassembly Corilagin-NPs was 82.7 ± 2.05 nm with less poly dispersive index (PDI), as shown by the DLS method (Fig. 2C). Admirable findings are associated with the formations of supramolecular nanoassembly Corilagin-NPs. The stability of the Corilagin, and Corilagin-NPs in PBS media was observed by measuring the particle size of the Corilagin, and Corilagin-NPs by dynamic light scattering. Polyplexes index (PDI), precisely Corilagin, and Corilagin-NPs, at an NPs ratio of 100:1 were organized and incubated for 30 min at 37°C in order to check broad polyplex formations (Fig. 2D-E). All the stability analysis were three times repeated.

3.2. In vitro Corilagin release profiles

As a traditional nucleosides analog, the main shortcomings of Corilagin as a chemotherapeutic agents contain half-life small plasma and fast cytokine deaminase deactivation. We also developed supramolecular nanoassembly Corilagin-NPs which could help as a reservoir to keep drugs safe and avoid the release of drugs into the general blood circulation, thereby slowing down Corilagin authorization from the human body condition. Observation of the controlled release of Corilagin-NPs by dialyzing against PBS at RT. As seen in Fig. 2F, Corilagin free was rapidly released from Corilagin-NPs and plateaued at 90.2 ± 2.9 compound releases after 25 hrs of incubation. In the other hand, Corilagin-NPs demonstrated continuous drug release. Our findings of in vitro drug leasing revealed a sluggish inhibition of the release of free CORILAGIN kinetics from the Corilagin-NPs, which is useful to enhance the half-life blood plasma of free Corilagin and enhancing drug delivery to cancer cells.

3.3. In vitro proliferation assay by MTT

After efficient fabrication of Corilagin-NPs, MTT experiments were conducted to determine the proliferation of Corilagin and Corilagin-NPs of cervical carcinoma cells, with CaSki and HeLa cancer cells. After treating with drugs for 24 hrs, the proliferation of the CaSki and HeLa cells was controlled and the dose dependent curvature shows the half inhibitory concentrations (IC_{50}) (Fig. 3A-C). Positively, associated to the free Corilagin, supramolecular nanoassembly displayed significantly increased cytotoxic effects in both cervical cancer cell lines tested. In half-inhibitory concentrations of CaSki cells, the IC_{50} is 11.12 ± 5.09 and 6.13 ± 1.29 for Corilagin and Corilagin-NPs respectively. In HeLa cells, the IC_{50} is 15.87 ± 2.18 , and 5.16 ± 2.80 for Corilagin and Corilagin-NPs, respectively. In addition, we examined the selectivity of the Corilagin and Corilagin-NPs shows remarkable proliferation against the CaSki and HeLa cells (Fig. 3C). The Corilagin-NPs shows remarkable proliferation against the CaSki and HeLa cells which may be due to the presence of the hydrophilic linoleic acid of π -conjugation chain. This may be attributed due to the hydrophobic chain of the linoleic acid which may efficiently penetrate the cell membranes. Several attempts will be made to establish the small molecule nano-assembled nanoparticles for

promising anticancer agents by introducing the various lipophilic spacers on the potential small molecules.

3.4. Cellular uptake efficiency of Corilagin-NPs

We cross-linked that the Corilagin-NPs make it possible to interact with CaSki and HeLa cervical tumor cells and ions, thereby enhancing intracellular absorption into the cervical cancer cell. A confocal laser scanning microscopy (LCSM) was used to test the localizations of subcellular of Corilagin-NPs in the CaSki and HeLa cervical cancer cell lines (Fig. 4). In the concentration 20 nM with Corilagin-NPs with different incubation times (10, 20 and 30 min) were labelled with the Dil fluorescence tracker (red color) and LysoTracker-Green (green color) for the interpretations, for this comparison the cells lysosome and the nucleus was labeled with the DAPI (blue color). The new yellow color fluorescents were paired with Dil, LysoTracker-Green and DAPI fluorescence in the CaSki and HeLa cervical cancer cell line, whereas Corilagin-NPs could be confined with lysosome during internalization.

3.5. Morphological examination by acridine orange/ethidium bromide (AO/EB)

Corilagin and Corilagin-NPs were tested using a fluorescent microscopic examination of acridine orange/ethidium bromide (AO/EB) stained in CaSki and HeLa cervical cancer cell lines indicative of morphological changes (Fig. 5). Usually nanoparticles causes cell death through the apoptosis and necrosis pathways. Ironically, after 24-hour treatment with their IC_{50} concentration of the Corilagin and Corilagin-NPs, Corilagin-NPs display a higher proportion of cell death by apoptosis pathway than free Corilagin treatments.

3.6. Morphological examination by Hoechst-33258 staining

Corilagin and Corilagin-NPs were tested using a fluorescent microscopic examination of Hoechst-33258 staining (nuclear staining) stained in CaSki and HeLa cervical cancer cell lines indicative of morphological changes (Fig. 6). Usually nanoparticles causes cell death through the apoptosis and necrosis pathways. Ironically, after 24-hour treatment with their IC_{50} concentration of the Corilagin and Corilagin-NPs, Corilagin-NPs display a higher proportion of cell death by apoptosis pathway than free Corilagin treatments.

3.6. Flow cytometry-confirmation of the apoptosis

Apoptosis can be used as a significant impairment to the growth of a tumor cells. The primary qualitative screening methods of the AO/EB and nuclear staining methods clearly shows the supramolecular nanoassembly induce the cell death through apoptosis mode. In this quantitative experiments displays the number of cancer cell death by the flow cytometry via staining of the FITC Annexin-V and propidium iodide (PI) in the CaSki and HeLa cervical cancer cells. Corilagin and Corilagin-NPs were tested using a

FITC Annexin-V with the propidium iodide (PI) examination in CaSki and HeLa cervical cancer cell lines (Fig. 7). Ironically, after 24-hour treatment with their IC_{50} concentration of the Corilagin and Corilagin-NPs, Corilagin-NPs with high percentage of the apoptosis than free Corilagin.

3.7. Hemocompatibility assay

Analysis of the relationship between nanoparticles and human blood erythrocytes using hemolysis assays is crucial to evaluating the blood viability of nanoparticles (Fig. 8). Free Corilagin and Corilagin-NPs have been shown to have excellent biocompatibility with human red blood cells, as shown in Fig. 8. The function of toxic substances appears to be nano-specific. According to IOS/Technical Report 7406, the hemolytic levels of nanoparticles or materials is limited to 5%. The release of erythrocytes by Corilagin-NPs was insignificant, suggesting that the nanoparticles had a marginal amount of toxicity and were thus safe for the cells.

4. Conclusion

We have effectively engineered and illustrated the lipophilic and fast metabolic Corilagin prodrugs for a more pharmacologically effective nano prodrug. We find out that supramolecular nanoassembly of Corilagin nanoparticles display improved controlled drug release and enhanced extracellular environment uptake to possibly resolve cancer cell aggregations by EPR. After a successful development, we tested the MTT of Corilagin and Corilagin-NPs against CaSki and HeLa cervical tumor cell lines. In addition, the morphological changes experiments such as acridine orange/ethidium bromide (AO-EB), Hoechst-33258 staining results shows that the supramolecular nanoassembly of Corilagin nanoparticles induce apoptosis in cervical cancer cells. Further, we have confirmed the apoptosis by flowcytometry techniques. The findings of Corilagin-NPs nanoparticles will invention possible application of cervical patents for better chemotherapy and potential clinical investigations.

Declarations

Conflict of the interest

None

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Figures

Figure 1

Graphical description of Corilagin-NPs affecting cervical cancer cells. (a) Structure of the Corilagin. Graphic design of the Corilagin prodrug formulation preparation process and can be nanoassembly in water by Corilagin-NPs displaying a possible chemotherapy outcome.

Figure 2

Characteristic assessment of Corilagin-NP. (a) TEM representation of Corilagin-NPs. The scale bar is 100 nm. (b) Hydrodynamic parameter Corilagin-NPs. (c) Hydrodynamic parameter diagram of Corilagin-NPs. D) the ability of Zeta to be investigated by a DLS review. E) In vitro controlled drug release profiles of Corilagin drugs from Corilagin-NPs. The solution contains Corilagin-NPs which have been dialyzed against PBS at 37°C.

Figure 3

In vitro proliferation of CaSki and HeLa cervical cancer cell lines along with non-cancerous NIH-3T3 cells. MTT assay was performed to examine the proliferation of the non-cancerous and cancer cells with different concentrations.

Figure 4

Cellular uptake efficiency of the CaSki and HeLa cervical cancer cells. Lysosomes labeled with subcellular localization of Corilagin-NPs in CaSki and HeLa cervical cancer cell lines at different incubation periods. Scale bar = 20 μ m.

Figure 5

Dual AO/EB fluorescent staining of CaSki and HeLa cervical cancer cell lines after treating with Corilagin and Corilagin-NPs with IC50 concentration for 24 hrs.

Figure 6

Nuclear staining of CaSki and HeLa cervical cancer cell lines after treating with Corilagin and Corilagin-NPs with IC50 concentration for 24 hrs.

Figure 7

Flow cytometry examination were performed to confirm the apoptosis of CaSki and HeLa cervical cancer cells. The CaSki and HeLa cells were treated with Corilagin and Corilagin-NPs with IC₅₀ concentration for 24 hrs and stained by FITC-Annexin V and PI for flow cytometry examination.

Figure 8

Hemocompatibility assay with various concentrations of Corilagin and Corilagin-NPs. The result of hemocompatibility assay shows that the remarkable hemocompatibility which displays that is precise bio-compatibility profiles for in vivo.

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