mPEG-icariin nanoparticles for treating myocardial ischaemia

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ABSTRACT

Icariin (ICA), a major active ingredient from Chinese medicine, has unique pharmacological effects on ischaemic heart disease. However, its hydrophobic property limits its administration and leads to poor efficacy. This work aimed to change its hydrophobic property and improve the treatment efficacy. We designed a new nano-drug to increase the ICA delivery. ICA was modified with hydrophilic polyethylene glycol monomethyl ether (mPEG) by a succinic anhydride linker to form a polyethylene glycol-icariin (mPEG-ICA) polymer. The structure of this polymer was identified by Fourier transform infrared spectroscopy and nuclear magnetic resonance spectroscopy. The content of ICA in the polymer was 32% as detected by ultraviolet spectrophotometry. mPEG-ICA nanoparticles, of 143.3 nm, were prepared by the dialysis method, and zeta potential was 0.439 mV by dynamic light scattering. The nanoparticles had a spherical shape on transmission electron microscopy. In media with pH 7.4 and 6.8, ICA release from mPEG-ICA nanoparticles after 72 h was about 0.78% and 64.05%, respectively, so the ICA release depended on the release media pH. On MTT and lactate dehydrogenase activity assay, mPEG-ICA nanoparticles could reduce cell damage induced by oxygen-glucose deprivation. Hoechst 33258 staining and TUNEL and Annexin V-FITC/PI double staining showed that ICA nanoparticles could increase the activity of H9c2 cardiomyocytes under oxygen-glucose deprivation conditions by decreasing apoptosis. ICA modified by hydrophilic mPEG could improve its efficacy.

Introduction

Ischaemic heart disease is a chronic disease requiring long-term treatment, so drug treatment is a critical treatment approach [1]. Studies have found that Chinese medicines such as flavonoids, astragalside and icariin (ICA) have a significant effect on ischaemic heart diseases [2–4]. ICA increases cardiovascular blood flow, promotes haematopoietic function and dilates blood vessels to reduce blood pressure, which has been widely studied recently [5–7]. ICA is extracted from the traditional Chinese medicine plant Epimedium brevicornu maxim (structure in Figure 1). However, ICA is limited in clinical applications because of its first pass elimination and poor water solubility and thus low bioavailability [8,9]. For full pharmacological advantages of ICA, ICA nanoparticles (NPs) have been heavily researched and designed [10,11]. The study of the preparation of ICA NPs has included ICA-loaded solid lipid NPs and ICA magnetic nano-liposome [12,13]. As compared with pure ICA, ICA nano-drugs can improve the in vivo bioavailability, so nano-preparations are an ideal choice for efficacious delivery of ICA [14]. As a foreigner, nano-drug preparation is easy to be eliminated during intravenous delivery by the reticular endothelial system before it acts [15].

Polyethylene glycol (PEG) is a highly versatile hydrophilic polymer commonly used to modify the surface of NPs or to constitute NPs as a hydrophilic section [16,17]. Surface PEGylation can reduce the adsorption and removal of NPs by macrophages during systemic circulation [18]. Therefore, PEGylated NPs are also commonly called “invisible” NPs; the long cycle in body is vital for drug delivery [19,20]. In addition, as a part of the drug delivery system, PEG implants new anticoagulant properties and functions such as the antiphagocytosis of macrophages [21]. PEGylated drugs, dissolved macromolecules with linear and flexible structures, tend to diffuse in the interstitial space of the heart, which helps the drug reach a perfusion defect or non-perfusion area [22]. Therefore, it is a good choice for the delivery to ischaemic sites.

We chose PEG monomethyl ether (mPEG), a derivative of PEG, with good hydrophilic properties and stable chemical structure, to graft to ICA in this study. Because of the low activity of the end hydroxyl, it must be activated by modification of mPEG. One way is to treat mPEG with carboxylation [23]. There are currently three ways: (1) nucleophilic substitution by using mPEG and halogenated acetic ester; (2) esterification by using mPEG and succinic anhydride; and (3)
oxidation of the end hydroxyl group of mPEG with oxidants. We chose succinic anhydride as the linker to graft mPEG, as well as ICA. NPs have a unique advantage in drug delivery of insoluble drugs, macromolecular drugs and gene therapy [24,25]. The NP is coupled with the hydrophilic mPEG micelle shell, so it is not easy to be absorbed by the reticular endothelial system because of the prevention of protein and cell adsorption [26]. As well, with the nanoscale particle size, the drug-carrying NPs in the targeted location can have better biomembrane penetration [27]. There is a similar permeability and retention function (EPR) in the ischaemic region of the myocardium as compared with tumour tissue [28]. mPEG of NPs, as a long cycle of drug delivery, can deliver the drug to the targeted position of myocardial ischaemia tissue. Therefore, ICA was designed as a type of mPEG NP to improve the bioavailability and for high efficacy of targeted treatment on myocardial ischaemia.

Amphiphilic polymer NPs, which belong to the nano-associative colloid system, are a new type of drug carrier with high drug-carrying capacity and unique distribution characteristics in vivo [29,30]. The polymer NPs self-assemble into the core-shell structure from two hydrophilic copolymers in water; the core section can be used as a container for hydrophobic drugs, and the shell can protect the drug, improve the stability of drugs, and achieve a slow-release effect [31,32]. We have studied the pharmacokinetics of ICA in vivo and have reported the method of linking hydrophilic molecules to produce amphiphilic conjugates [33]. With this knowledge, we designed the hydrophobic section of ICA and the hydrophilic section of carboxylated mPEG, and combined the two sections by esterification. In aqueous solution, mPEG-ICA NPs were formed by partial connection to form a hydrophilic PEG shell and ICA hydrophobic core. NPs prepared with this method can increase the solubility of ICA in aqueous solution. The ICA release from mPEG-ICA NPs was studied in media at pH7.4 and 6.8. Furthermore, to investigate the effect of mPEG-ICA NPs on ischaemic myocardia, we set up the oxygen-glucose deprivation model and detected cell viability and apoptotic rate.

Materials and methods

Materials
ICA (purity = 95%) was purchased from Saan Chemical Technology Co. (Shanghai); mPEG was from Sigma-Aldrich (Shanghai); N-hydroxysuccinimide (NHS) was from Shanghai Ruiyong Biotechnology Co. (Shanghai); 4-dimethylaminopyridine (DMAP) was from Aladdin Reagent Co. (Shanghai). Other analytical pure reagents were from Sinopharm Chemical Co. (Shanghai).

Methods

Synthesis of mPEG-ICA conjugates

Synthesis of mPEG-COOH. An amount of 5.00 g mPEG, 0.40 g succinic anhydride (SA), and 0.50 g DMAP (molar ratio 1:1.5:1.5) was added to a 250-ml flask for stirring at 60°C for 2 h to remove water by using dichloromethane; the solvent was then removed by rotary evaporation at 35°C for 12 h (Figure 2). Then 150 ml ether was added into the white powder obtained and filtered out. The powder was rinsed with 50 ml ether three times [34]. It was kept at room temperature and atmospheric pressure for half a day until no ether residue appeared. The powder was dissolved with 50 ml secondary distilled water, dialyzed in 2000 dialysis bag for 48 h with the water constantly replaced. Dissolved SA was removed and undissolved SA was filtered out. The carboxylated mPEG (mPEG-COOH) was obtained by freeze-drying the filtrate.

Synthesis of mPEG-ICA polymer. An amount of 0.37 g mPEG-COOH, 0.024 g NHS, and 0.026 g DMAP was added in a 250-ml flask, with 10 ml dimethyl sulfoxide (DMSO) as a solvent and stirred for 12 h at room temperature to activate carbonyl group. Then 100 mg standard ICA was added, and 5 ml DMSO was added to dissolve it fully. Then the standard sample was added and stirred for 48 h under the protection of nitrogen gas at room temperature. After continuous dialysis with secondary distilled water to eliminate the DMSO odour, the dialyzed substance was freeze-dried for 48 h to obtain the yellowish product, the mPEG-ICA polymer (0.1868 g).

Fourier transform infrared (FTIR)
The standard samples of ICA, mPEG-COOH and mPEG-ICA were ground into powder in an agate abrasive bowl and mixed with dry KBr powder; each of these was pressed into a thin film for FTIR scanning, with a range of 4000–400 cm⁻¹.

Proton nuclear magnetic resonance (¹H-NMR)
ICA, mPEG-COOH, and mPEG-ICA were dissolved in DMSO-d₆ and added into the sample tube to be recorded on a 500 MHz NMR spectrometer.

Ultraviolet (UV-Vis) spectroscopy
An amount of 4.0 mg standard sample of ICA was diluted to 25 ml with methanol, shaken and dissolved completely. Amounts of 0.3, 0.5, 1.0, 1.5, 2.0 and 2.5 ml of the standard solution were kept in 25-ml flasks. The UV spectrophotometer (Beijing Leibertke instrument Co.) was set at 270 nm
(maximum absorption wavelength), and methanol was used as a blank control to measure the absorbance; the data were recorded for linear regression. An amount of 4.3 mg mPEG-ICA polymer was diluted to 25 ml with methanol. Three samples of the mPEG-ICA solution (2.0 ml) were prepared and measured at 270 nm. The data were collected for calculating the average ICA content of the mPEG-ICA polymer.

**Preparation of mPEG-ICA NPs**

An amount of 5 mg mPEG-ICA was weighed in a small tube, dissolved in 2 ml DMSO, then shaken in the water bath for 5 min at 37 °C. An amount of 5 ml secondary distilled water was added to a small beaker, placed in a small stirrer, then mPEG-ICA dissolved in DMSO was slowly added and stirred on a magnetic stirrer for 15 min at room temperature. The reaction was dialyzed in water for 24 h by using a dialysis bag (molecular weight cut-off 35 kDa); within this time, water was continuously changed to dialyze the DMSO solvent clearly and filtered to obtain a mPEG-ICA polymer NP solution.

**Characterization of mPEG-ICA NPs**

Dynamic light scattering (DLS; Zetasizer 3000 HS, Malvern Instruments, Malvern, UK) was used to determine the size distribution and zeta potential of mPEG-ICA NPs, which was newly prepared or freeze-dried in distilled water after the dispersion of NP solution into the colorimetric cup. A drop of mPEG-ICA NP (1.0 mg/ml) solution was dropped onto a copper grid with a carbon support film, and the filter paper was drained. Grids were placed in a desiccator, then 2% (w/w) phosphotungstic acid (2%) was added, which was negative after drying naturally, and

**Figure 2.** The chemical synthesis of mPEG-ICA.

**Figure 3.** FTIR spectra of ICA (A), mPEG-COOH (B), and mPEG-ICA (C).
transmission electron microscopy (TEM) was used to observe morphological characteristics at acceleration voltage 80 kV.

**Drug release in different release media**

An amount of 2 ml mPEG-ICA NP solution was placed into a dialysis bag (4–8 kDa, molecular weight cut-off) and dialyzed in 25 ml phosphate buffered saline (releasing media) at pH 7.4 and 6.8 at 37 °C. Then, 2 ml releasing medium was collected for sampling and replaced with an equal volume of the fresh solution at pre-defined time intervals (Tn, n = 0, 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 h). The absorbance was measured by ultraviolet spectroscopy. The percentage rate of ICA release (Q%) was calculated as described [35], and three samples were measured to calculate average values of drug release.

**Cell testing experiment**

**Cell culture**

H9c2 cardiac cell lines (rat H9c2 [2–1]) were purchased from the cell Library of the Chinese Academy of Sciences, Shanghai, and cultured in DMEM supplemented with 10% foetal bovine serum (FBS) at 37 °C in a 5% humidified atmosphere [36]. H9c2 cells were routinely grown to ~ 80% confluence of the bottom of 10-cm Petri dishes, then subcultured with 0.25% trypsin (Gibco) the cells were seeded in corresponding dishes or 96-well plates for the following studies.

**Oxygen-glucose deprivation (OGD) model and treatment**

To mimic the ischaemic condition in vitro, H9c2 cells were exposed to oxygen-glucose deprivation as follows: ischaemia simulation solution (mmol L⁻¹: NaCl 98.5, MgSO₄ 1.2, KCl 10, CaCl₂ 1.0, sodium lactate 40 and HEPES 20, pH 6.8, 37 °C) was pre-saturated under 95% N₂+5% CO₂ for 1 h, then the culture medium (DMEM supplemented with 10% FBS) was replaced with ischaemia simulation solution and cells were transferred to a hypoxia chamber (95% N₂+5%CO₂) for 4 h. The cells were randomly divided into four groups as follows: (1) normal culture group: culture with DMEM supplemented with 1% FBS for 4 h in a hypoxia chamber (95% N₂+5%CO₂); (2) OGD group: culture with ischaemia simulation solution (deoxygenated/glucose-free solution) in the hypoxic incubator (95% N₂+5% CO₂) for 4 h; (3) OGD + ICA group: incubation with 40 μmol L⁻¹ ICA, and the other conditions the same as for the OGD group; (4) mPEG-ICA NP group: incubation with 40 μmol L⁻¹ mPEG-ICA NPs, and the other conditions were same as above. The ICA was dissolved in DMSO and diluted with ischaemia simulation solution to the final concentration (DMSO:solution = 1:800). The other groups were dissolved in DMSO (1:800).

**Cell viability by MTT assay**

Cell viability was detected by MTT assay, then the same amount of cells was seeded on 96-well plates. At the end of OGD treatment, 0.5 mg/mL MTT was added into the medium

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*Figure 4.* ¹H NMR hydrogen spectra for mPEG-COOH and mPEG-ICA.
for 4 h at 37 °C, then the medium was removed, and 150 µL DMSO was added to each well to dissolve the formazan crystals. The optical density (OD) was measured at 570 nm by using a microplate reader. The survival ratio was expressed as a percentage of the control.

**Lactate dehydrogenase (LDH) release assay**

To assess cell injury induced by oxygen-glucose deprivation, we detected LDH release in the medium. Briefly, the culture medium was collected in each group at the end of treatment, and LDH activity was detected by using an LDH
detection kit (Beyotime biotechnology) according to the manufacturer’s instructions. Finally, the absorbance value was measured at 492 nm with a spectrometer.

**Hoechst 33258 staining**

The extent of apoptosis was evaluated by the alteration in nuclear morphology by using the nucleus-specific dye Hoechst 33258. Cells were cultured in 6-well plates, then after treatment, incubated with Hoechst 33258 at 37°C for 15 min and rinsed twice with PBS, then immediately observed under a fluorescence microscope (Leica, Heidelberg, Germany). Apoptosis index = apoptotic nuclei amount/total nuclei amount × 100% [37].

**Flow cytometry**

The apoptotic rate of H9c2 cells was detected by flow cytometry by AnnexinV-FITC/PI double staining. Briefly, at the end of treatment, cells were collected by centrifugation at 1500 rpm for 10 min, then resuspended in 100 μL binding buffer. AnnexinV-FITC was added for 15 min and PI for 5 min at room temperature in darkness, then diluted with 400 μL binding buffer, and analyzed by flow cytometry.

**TUNEL assay**

Apoptosis was assessed by Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay. H9c2 cells were cultured in 24-well culture plates and treated, then washed with PBS and fixed with 4% polyformaldehyde. An amount of 1% Triton x-100 was used to increase the cell membrane permeability. Cells were washed with PBS three times and stained by using the one-step TUNEL apoptosis assay kit (Beyotime biotechnology) according to the manufacturer’s protocol, and apoptosis was observed on fluorescence microscopy. The cell apoptosis rate in each group was expressed as the percentage of green fluorescence nucleus (TUNEL) in the total cell nucleus (blue) (nuclei were stained with DAPI). Three independent experiments were performed.

**Results**

**FTIR spectra**

From the infrared spectra for the standard sample of ICA, the main characteristic peaks occurred at 3440, 2960, 2840, 1650, 1600, 1350, 1260 and 1180 cm⁻¹; 3440 cm⁻¹ was the stretching vibration peak of the phenolic hydroxyl group in ICA (Figure 3). Because of a large number of phenolic hydroxyl groups in the molecule, an association phenomenon occurred, which resulted in a strong and broad absorption peak. The spectrum 1650 cm⁻¹ was a C=C stretching vibration peak in ICA, 1600 cm⁻¹ was a C=O stretching vibration peak, and a strong peak was formed by the conjugation effect of C=C and C=O; 1260 cm⁻¹ was a C—O extension vibration peak.

From the mPEG-COOH spectra, a clear methylene peak could be seen, and a monomer was the stretching vibration peak of the carboxyl group (—COOH) at 1720 cm⁻¹; 1620 cm⁻¹ was the C=O stretching vibration peak. The peak of 1740 cm⁻¹ next to it was the stretching vibration absorption peak of the ester bond (—C=O—), which was more obvious, which indicates that the carboxylation of mPEG was successful.

From the mPEG-ICA spectra, the stretching vibration absorption peaks of two ester bonds (—C=O—) were at 1700 and 1740 cm⁻¹. As compared with the mPEG-COOH spectra, there was an excess C=C stretching vibration peak at 1650 cm⁻¹, so the esterification synthesis of ICA and mPEG-COOH to form mPEG-ICA was successful.

**¹H NMR spectroscopy**

From the mPEG-ICA hydrogen spectra, 12.6 ppm was a phenolic hydroxyl group of ICA due to the presence of a C=O strong absorption electron group moving toward a low field (Figure 4). A hydrogen peak of a benzene ring appeared near 7.5 ppm, and the 3.5 ppm peak area was very large, inferred as the —CH₂—O—hydrogen peak in polymer mPEG. The hydroxyl hydrogen signal on ICA was 0–2 ppm, and 1.7 ppm was the double bond hydrogen peak in ICA. The characteristic peaks of mPEG-COOH and ICA appeared correspondingly in mPEG-ICA, which demonstrated the successful synthesis of PEG-ICA.

**ICA content in the mPEG-ICA polymer**

The ICA content in the mPEG-ICA polymer could be calculated by ultraviolet spectrophotometry. The standard curve of ICA concentration was established, and the ICA content in the polymer was obtained by calculating the drug concentration by the standard curve. With sample absorbance 0.224660, the ICA content was 0.0045 mg/ml and sample content was 0.01376 mg/mL, for an ICA content in the mPEG-ICA polymer of about 32%.

**Particle size, zeta potential and TEM of mPEG-ICA polymer NPs**

The average particle size of the mPEG-ICA NPs was about 143.3 nm, within a suitable size range, and the particle size...
The distribution was relatively narrow; the polymer dispersibility index (PDI) was 0.226 (Figure 5(A)). The smaller the PDI, the more uniform the distribution of mPEG-ICA NPs. The zeta potential of mPEG-ICA NPs was 0.439 ± 0.258 mV, which was close to neutral (Figure 5(B)). TEM revealed that the mPEG-ICA NPs had a spherical shape (Figure 5(C)).

ICA release from mPEG-ICA NPs

The release of ICA greatly depended on the pH value of the releasing media (Figure 6). The cumulative release amount in pH 7.4 for 72 h was only (0.78 ± 0.02)% close to 0. At pH 6.8, the cumulative release during 72 h reached (64.05 ± 1.02)% and the ICA release increased at each time point. At pH 7.4, mPEG-ICA NPs were stable, with no ICA release. Under the acidic conditions of pH 6.8, part of the mPEG-ICA may have been depolymerized or the mPEG-ICA molecules broken in the NPs, which led to the gradual release of ICA and the rapid increase in ICA content. The release characteristics of mPEG-ICA NPs are beneficial for local treatment of myocardial ischaemic injury because local lesions show weak acidity [38].

Effect of mPEG-ICA on cell viability and LDH release in H9c2 cells exposed to OGD

To assess the effect of mPEG-ICA on cardio protection, MTT assay was used to evaluate cell viability of H9c2 cells. After cells were exposed to OGD for 4 h, cell viability was decreased to
47.33 ± 7.40% (normal culture considered 100%) (Figure 7). As compared to cells with OGD alone, OGD-treated cells with mPEG-ICA NP and ICA treatment showed significantly improved viability (59.97 ± 8.3% and 58.07 ± 5.92%, \( p < 0.05 \)), with no significant difference between the two groups.

We also detected LDH release. LDH release was increased in the medium after H9c2 cells were exposed to OGD as compared with normal culture. mPEG-ICA NP and ICA treatment stopped the OGD-increased LDH release (Figure 8).

**Effect of mPEG-ICA on cell apoptosis induced by OGD**

Hoechst33258 was chosen to stain nuclei. Typical apoptotic cells had nucleus condensation, breakage and fragmentation. In the OGD group, the number of apoptotic nuclei increased to 31.62 ± 7.80% (\( p < 0.05 \)) as compared with normal culture (2.70 ± 3.05%). Apoptosis rate decreased to 15.57 ± 2.58% and 22.88 ± 8.65% with mPEG-ICA NP and ICA treatment, respectively, with a significant difference between the two groups (Figure 9). Furthermore, cells were exposed to OGD and were double stained by AnnexinV/PI, and analyzed by flow cytometry. As compared with OGD treatment alone (46.68 ± 10.47%), the death rate of early and late apoptotic cells was reduced with mPEG-ICA NP and ICA treatment (19.72 ± 11.37%, 20.37 ± 8.75%) (Figure 10). We used apoptotic marker-TUNEL staining to observe cell DNA fragmentation or DNA damage. The results agreed with the above two methods (Figure 11).
Discussion

ICA has been found effective for treating ischaemic heart disease; however, the clinical application of ICA is limited because of its initial effect and poor water solubility [39]. Research has shown that drug-delivery characteristics could be improved by combining ICA with nano-carrier technology [40,41]. However, in this study, we improved the drug-delivery characteristics of ICA with a novel method in which mPEG was modified by ICA via a chemical link to form polymer NPs.

The method is highly feasible and has many advantages: First, mPEG is non-toxic, a non-irritant, and widely used in various pharmaceutical preparations. Also, the water solubility of insoluble ICA can be significantly improved by modification with mPEG, and the cell toxicity reduced [42]. Second, the synthesis route of the mPEG-ICA polymer is simple and easy to perform. Also, mPEG-ICA NPs can provide some strategies for further study of other nano-preparations of ICA. Third, a hydrophobic cavity appears after the spontaneous formation of mPEG-ICA polymer NPs, and the cavity can be further loaded with ICA or other anti-myocardial ischaemia drugs for synergistic treatment of myocardial ischaemia.

In this experiment, we designed mPEG-ICA with a hydrophilic section of mPEG and hydrophobic section of ICA and used a dialysis method to form stable NPs, which led to self-assembly by hydrophobic action. The ICA release from mPEG-ICA NPs was suitable for treatment of myocardial ischaemia. At pH 7.4, little ICA was released, which implies that the mPEG-ICA NPs injected into the blood will be stable in the blood circulation and not delivered into tissue. At pH 6.8, ICA release increased, so use of mPEG-ICA NPs can lead to localized release in lesions of ischaemic cardiomyopathy disease, with high efficacious exploitation of drug delivery. Our mPEG-ICA conjugates self-assembled into mPEG-ICA NPs in a dialysis bag (4–8 kDa, molecular weight cut-off) by the dialysis method.

mPEG-ICA can form a self-assembled NP successfully but not leak from the dialysis bag, even if the molecular weight of mPEG-ICA was about 3 kDa. Therefore, the self-assembled behaviour of mPEG-ICA was due to a strong force from the intermolecular process, which effectively avoided the leakage of the mPEG-ICA molecule. It is vital to clarify the ICA release in media of pH 7.4 and 6.8. At pH 7.4, the self-assembly force of mPEG-ICA NPs was the same as during the formation

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Figure 11. Detection of apoptotic cell nuclei by TUNEL staining. (A) H9c2 tunnel staining; green fluorescence indicates apoptotic cells, blue fluorescence nuclear DAPI staining; (B) Quantification. Data are mean ± SD. * p < 0.01 compared with normal culture, **p < 0.01 compared with OGD.
period of NPs. At pH 6.8, the acidic environment may destroy the self-assembled force of NPs, which led to the NPs depolymerizing and mPEG-ICA release into the released media. Also, with prolonged time, the ester bond of mPEG-ICA in the portion of NPs may be fractured in the acidic environment and ICA released as a free drug. Therefore, the ICA release increased at pH 6.8. Also, ICA release was faster than the drug release of other NPs assembled by similar conjugates consisting of nanomaterials and a drug with a chemical link [43,44]. The ICA release may be from ICA and also mPEG-ICA. The release characteristic of mPEG-ICA is vital for use in cardiomyopathy disease treatment.

Results of the MTT assay and LDH release demonstrated that mPEG-ICA could decrease cell damage induced by OGD and that mPEG-ICA NPs could release ICA completely from particles. Previous studies have demonstrated that cardiomyocyte ischaemia leads to cytochrome C release from dysfunctional mitochondrial into the cytosol, then cytochrome C initiates apoptosis, which results in functional cell loss and decreased heart function [45,46]. Therefore, anti-apoptosis is a new strategy for treating ischaemic cardiomyopathy. Traditional Chinese medicine, such as berberine, tetrabenazine and resveratrol, can improve heart function and reverse cardiac remodelling by reducing apoptosis [47,48]. We detected the effect of mPEG-ICA on cell apoptosis by Hoechst 33258 and TUNEL staining and flow cytometry. mPEG-ICA could significantly decrease cell apoptosis induced by OGD. Furthermore, the anti-apoptosis effect of mPEG-ICANPs was better than with free ICA. Therefore, mPEG-ICA NPs had a cardio protection function against ischaemia and may be a new drug preparation for preventing cardiac ischaemia. This in vitro experiment revealed a new type of mPEG-ICA NPs with effects on myocardial ischaemia, which may be beneficial for treating myocardial ischaemic damage. There is room for improvement in our research. The content of ICA in mPEG-ICA NPs could be enhanced and the particle size of mPEG-ICA NPs could be controlled by improving the preparation methods, which is vital for in vivo study.

Conclusions

mPEG-ICA polymer was synthesised successfully for a new type of mPEG-ICA NP created via nanotechnology to change the hydrophobic drug ICA into a water-soluble nano-drug. ICA release from mPEG-ICA NPs was increased in weakly acidic media but barely in neutral media, which is meaningful for treating ischaemic cardiomyopathy. Nano-ICA could increase cell viability and decrease cell apoptosis induced by oxygen-glucose deprivation, so the nano-ICA would have good therapeutic prospects for ischaemic cardiomyopathy.

Acknowledgements

The authors thank Qiufang Zhang for funding acquisition and ZQF for editing the manuscript.

Disclosure statement

The authors declare no conflict of interest.

Funding

This project was supported by the Health and Family planning commission of Hunan Province research project (No.B2017073) to Xiaojun Tao; National Natural Science Foundation of China (Nos.81303254, 81641140) and Hubei Province health and family planning scientific research project (No.WJ2017M214) to Qiufang Zhang; Changsha science and Technology Bureau (No. K1303022-31) to Chunli He.

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