Polymeric nanoparticles developed by vitamin E-modified aliphatic polycarbonate polymer to promote oral absorption of oleanolic acid

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ABSTRACT
Oleanolic acid (OA) exhibited good pharmacological activities in the clinical treatment of hypoglycemia, immune regulation, acute jaundice and chronic toxic hepatitis. However, the oral delivery of OA is greatly limited by its inferior water solubility and poor intestinal mucosa permeability. Herein, we developed a novel polymeric nanoparticle (NP) delivery system based on vitamin E modified aliphatic polycarbonate (mPEG-PCC-VE) to facilitate oral absorption of OA. OA encapsulated mPEG-PCC-VE NPs (OA/mPEG-PCC-VE NPs) showed uniform particle size of about 170 nm with high drug loading capability (8.9%). Furthermore, the polymeric mPEG-PCC-VE NPs, with good colloidal stability and pH-sensitive drug release characteristics, significantly enhanced the in vitro dissolution of OA in the alkaline medium. The in situ single pass intestinal perfusion (SPIP) studies performed on rats demonstrated that the OA/mPEG-PCC-VE NPs showed significantly improved permeability in the whole intestinal tract when compared to OA solution, especially for duodenum and colon. As a result, the in vivo pharmacokinetics study indicated that the bioavailability of OA/mPEG-PCC-VE NPs showed 1.5-fold higher than commercially available OA tablets. These results suggest that mPEG-PCC-VE NPs are a promising platform to facilitate the oral delivery of OA.

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1. Introduction

Oral delivery is considered to be a preferred route for drug administration because of its convenience, painless administration, and high compliance with patients especially those in need of chronic therapies [1,2]. Drugs can maintain a sustained drug concentration in the circulation to increase the therapeutic efficiency [1]. However, multiple barriers hinder the oral administration of drugs due to their poor bioavailability, including physicochemical properties of drugs, physiological barriers in GI tract and biochemical barriers in GI tract [3]. At present, a wide range of oral drug delivery strategies to overcome the multiple barriers have been developed to enhance the stability of drugs, extend the GI tract residence time of drugs and carriers and promote the transport of drugs through the membrane of GI tract [2,3].

Oleanolic acid (OA) is a bioactive pentacyclic triterpenoid compound extracted from the leaves of Olea europaea L. (also known as olean fruit) and clove, swertia mileensis, prunella vulgaris and jujube [4–6]. It is widely used in the therapy of hepatoprotective, anti-oxidation, two-way immune regulation, hypolipidemic, hypoglycemic, anti-tumor and acute jaundice and chronic toxic hepatitis in clinical due to high pharmacological activity and low side-effect [4,5,7–11]. OA belongs to the Class IV in Biopharmaceutics Classification System (BCS), with poor solubility and permeability. Therefore the inferior aqueous solubility and poor permeability across the intestinal tract [12], and accompanying low bioavailability [13] provide a daunting obstacle to OA wide appliance.

In order to facilitate oral delivery of OA, several attempts have been made to improve its bioavailability, such as nanosuspensions [14–16], β-cycodextrin inclusion compounds, self-nanoemulsified formulations [17], phospholipid complexes [18–20], solid dispersions [21,22], and nanoparticles (NPs) [23,24]. Among them, polymeric NPs represent as a wide-applied drug delivery system by feat of better stability, versatile functionality and negligible adverse effects [25].

In the present study, a novel reported vitamin E modified aliphatic polycarbonate (mPEG-PCC-VE) polymer was employed to prepare NPs for oral delivery of OA. The prepared OA encapsulated mPEG-PCC-VE NPs (OA/mPEG-PCC-VE NPs) were characterized by a series of parameters including particle size, transmission electron microscope (TEM), differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD), colloidal stability and dissolution profile. In addition, the in situ single pass intestinal perfusion (SIP) and in vivo pharmacokinetics of the OA/mPEG-PCC-VE NPs were also evaluated. Our results clearly indicate a significant enhancement of permeability in the whole intestinal tract and an improved oral bioavailability of OA in the form of OA/mPEG-PCC-VE NPs as compared to commercial tablets after oral administration in rats.

2. Materials and methods

2.1. Materials

OA was purchased from Wuhan Dahua Pharmaceutical Co., Ltd. (Wuhan, Hubei). The chemical synthesis of mPEG-PCC-VE has been reported in our previous study [26]. HPLC grade of tetrahydrofuran was purchased from Concord Technology (Tianjin) Co., Ltd. (Tianjin, China). H3PO4 (HPLC grade) was obtained from Tianjin Kemiu Chemical Reagent Co., Ltd. (Tianjin, China). Methanol of HPLC grade was purchased from Thermo Fisher Scientific (Shanghai, China).

2.2. Preparation of OA/mPEG-PCC-VE NPs

The OA/mPEG-PCC-VE NPs were prepared by the emulsion solvent evaporation method. Briefly, OA was dissolved in tetrahydrofuran, then 1 ml resultant solution was added in 10 mg mPEG-PCC-VE. After stirring at room temperature, the mixture was dropped into water slowly and stirred. Then the mixture was sonicated by a probe-type sonifier (JY92-2D, Scientz, China) for 10 min in ice bath, and organic solvents were removed in a rotary evaporator under vacuum. After centrifuging for 10 min at 13000 rpm, the upper layer was filtered through a 0.45 μm filter membrane, then the OA/mPEG-PCC-VE NPs were obtained.

2.3. Characterization of OA/mPEG-PCC-VE NPs

2.3.1. Particle size and zeta potential

The particle size and size distribution of the prepared OA/mPEG-PCC-VE NPs were measured with dynamic light scattering (DLS) method by a Malvern Instruments Zetasizer (Nano ZS, Malvern Co., UK), and the measurements were repeated in triplicate.

2.3.2. Morphology

The morphology of OA/mPEG-PCC-VE NPs was observed by transmission electron microscope (TEM) (H-600, Hitachi, Japan). The particles were stained by 1.0% (w/v) phosphotungstic acid for 3 min on a copper grid stabilized with carbon support film.

2.3.3. DSC

DSC curves of OA, blank carrier material (mPEG-PCC-VE), physical mixture of OA and mPEG-PCC-VE were obtained using a DSC1 STAR® system (Mettler Tolelo, Switzerland). The samples (3.0–5.0 mg) were weighted accurately, sealed into aluminum pans separately and heated from 20 to 340 °C at a heating rate of 10 °C/min. Plots of weight versus temperature were recorded. The DSC results were analyzed using the STARE software.

2.3.4. XRPD

XRPD analysis was performed on a D\textit{Max}-2400 X-Ray powder diffractometer (Rigaku, Japan) at ambient temperature. Monochromatic Cu-Kα radiation (λ = 1.5406 Å) was used in the 2θ angle range from 3° to 50° with a step width of 0.05° at a voltage of 45 kV and current of 30 mA.

2.4. Colloidal stability of OA/mPEG-PCC-VE NPs

To investigate the colloidal stability of OA/mPEG-PCC-VE NPs in intestinal fluids, the prepared NPs were incubated at 37 °C with gentle shaking for 48 h. At regular intervals, the average particle size was determined by Malvern Instruments Zetasizer.
2.5. Determination of drug encapsulation efficiency and drug loading

2.5.1. RP-HPLC analysis
A Waters HPLC system including Pump e2695 Separations Module and Waters 2489 UV/Visible Detector (Waters Corp., Milford, MA, USA) was used to determine the concentrations of OA. A COSMOSIL 5 C18-AR-II column (150 mm × 4.6 mm, 5 μm) was used for the separation. Mobile phase composed of methanol and purified water containing 0.1% phosphoric acid (90:10, v/v) was used at a flow rate of 1.0 ml/min. The column temperature was maintained at room temperature. The wavelength of the UV detector was set at 210 nm. The calibration curve of OA concentrations (C, 4.09–204.5 μg/ml) versus integrated area (A, mAU) was plotted and the linear fit $A = 9238.8C+4458.3$ was obtained with a correlation coefficient of 0.9998.

2.5.2. Encapsulation efficiency and drug loading
OA/mPEG-PCC-VE NPs were dissolved and diluted with methanol and dispersed by ultrasound, which disrupted the NP structure to release OA. The drug loading (DL) and encapsulation efficiency (EE) of OA in the prepared NPs were evaluated by determining the encapsulated OA using the RP-HPLC method. Each measurement was carried out in triplicate, and the EE and DL of NPs were calculated by following equations:

$$EE(%) = \frac{S}{T} \times 100\%$$

$$DL(\%, w/w) = \frac{W_{OA}}{W_{Nano}} \times 100\%$$

where S is the amount of OA in the centrifugal supernatant, T is the total amount of OA added in the preparation, $W_{OA}$ is the weight of OA in the freeze-dried OA/mPEG-PCC-VE NPs, $W_{Nano}$ is the weight of the freeze-dried OA/mPEG-PCC-VE NPs.

2.6. In vitro drug release
The in vitro release performance of OA/mPEG-PCC-VE NPs was determined on a dissolution apparatus according to the paddle method described in the China pharmacopoeia (2015 edition). The dissolution medium was 900 ml deionized water containing 0.3% sodium dodecyl sulfate (SDS) [27]. The paddle speed was set to 100 rpm and the water bath temperature was maintained at 37 ± 0.5 °C. Samples (3 ml) of dissolution medium were withdrawn at 5, 10, 15, 20, 30, 45, 60, 90 and 120 min, and replaced with the same volume of fresh medium. The withdrawn samples were then filtered by 0.1 μm filter membrane and analyzed by HPLC at 210 nm. The dissolution data was modeled by DDSolver software [28].

2.7. In situ SPIP study of OA/mPEG-PCC-VE NPs
Healthy male Sprague–Dawley rats were obtained from the animal center of Shenyang Pharmaceutical University. The animal studies were approved by the Shenyang Pharmaceutical University Animal Care and Use Committee. Sprague–Dawley rats were fasted overnight with having access to water before perfusion experiment. After intraperitoneal injection of 20% urethane (1.0 g/kg), rats were restrained on a warming pad to keep body temperature. Then a gentle incision along mediaventral line was opened, and intestinal segments including duodenum, jejunum, ileum, and colon were recognized and pulled out carefully. Each intestinal segment was chosen about 10 cm, rinsed with freshly saline and balanced at a constant flow rate (Q) of 0.2 ml/min using peristaltic pump. The OA/mPEG-PCC-VE NPs were diluted to a final concentration equivalent to 20 μg/ml of OA. The intestinal perfusion lasted for 90 min, and all perfusion solution was collected. Finally the animal was sacrificed and the length (l) and radius (r) of the infused segments were measured precisely. The absorption rate constant ($K_a$) and apparent absorption coefficient ($P_{app}$) of OA was calculated by gravimetric method according to following equations:

$$K_a = \left(1 - \frac{C_{out}}{C_{in}} \right) \frac{V_p}{V}$$

$$P_{app} = -\frac{Q \cdot \ln \left( \frac{C_{out}}{C_{in}} \right)}{2\pi rl}$$

where $C_{out}$ is the OA concentration in the receptor tube, $V_{out}$ is the OA perfusion solution volume in the receptor tube, $C_{in}$ is the OA concentration in the donor solution, $V_{p}$ is the OA perfusion solution volume in the donor solution, Q is the perfusion flow rate, r is the intestinal radius and l is the length of infusion segment.

2.8. In vivo pharmacokinetic study
To evaluate the oral bioavailability of OA/mPEG-PCC-VE NPs, an in vivo pharmacokinetic study was carried out on Sprague–Dawley rats (200–250 g) according to the Guide for Care and Use of Laboratory Animals of Shenyang Pharmaceutical University. Sprague–Dawley rats were randomly divided into two groups, and the animals were fasten overnight with free access to water prior to the experiments. The suspensions of OA commercial tablets (20 mg/kg) and OA/mPEG-PCC-VE NPs (12 mg/kg) were administrated orally to rats, respectively. Blood samples (0.3 ml) were collected at 10 min, 20 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h and 24 h from the retro-orbital plexus under mild anesthesia after intragastric administration. Plasma was obtained by centrifuging the blood samples at 13000 rpm for 10 min and stored at −20 °C. The concentrations of OA in rat plasma were determined by a validated UPLC–MS–MS method.

The main pharmacokinetic parameters were calculated using DAS 2.1 software, and the relative bioavailability ($F_{rel}$) was calculated according to following equation:

$$F_{rel}(\%) = \frac{AUC_{nano} \cdot D_{CTs}}{AUC_{CTs} \cdot D_{mps}} \times 100\%$$

where $AUC_{nano}$ is the area under curve of the prepared NPs, $AUC_{CTs}$ is the area under curve of the commercial tablets, $D_{mps}$ is the dosage of the prepared NPs and $D_{CTs}$ is the dosage of the commercial tablets.
3. Results and discussion

3.1. Preparation of OA/mPEG-PCC-VE NPs

Emulsion solvent evaporation method was used to prepare the OA/mPEG-PCC-VE NPs at a drug-to-carrier ratio of 1:1.5 (w/w). After the performance of the hydration temperature was set at 25 °C, ultrasonic power 200 W and ultrasonic time 10 min, we can obtain NPs of uniform size distribution, satisfactory EE (71%) and DL (8.9%).

The particle size and PDI value of OA/mPEG-PCC-VE NPs were evaluated by a dynamic light scattering system. As shown in Fig. 1A, the mean particle diameter of OA/mPEG-PCC-VE NPs was 165.06 ± 1.08 nm, and the PDI value was 0.131 ± 0.058, indicating a narrow size distribution.

In addition, the stability of OA/mPEG-PCC-VE NPs in terms of size and PDI was shown in Fig. 2, which indicated that OA/mPEG-PCC-VE NPs were stable when stored at 37 °C for 42 h. However, PDI of OA/mPEG-PCC-VE NPs increased obviously when stored for 48 h due to some of the particles breaking and reaggregating.

3.2. Characterization of OA/mPEG-PCC-VE NPs

TEM was used to observe the morphology of OA/mPEG-PCC-VE NPs. As shown in Fig. 1B, the TEM images directly visualized the spherical structures of OA/mPEG-PCC-VE NPs.

The DSC curves of OA, mPEG-PCC-VE (Blank), OA and mPEG-PCC-VE complex (Physical mixture) and OA/mPEG-PCC-VE NPs were shown in Fig. 3. A sharp endothermic peak at 311.2 °C for the crude OA, indicative of its anhydrous and crystalline state.

However, the endothermic peak of OA disappeared in the DSC curve of OA/mPEG-PCC-VE NPs, indicating that OA existed in the NPs as amorphous state.

XRPD pattern was exploited to assess the crystal form of OA in OA/mPEG-PCC-VE NPs. The XRPD patterns of OA, Blank, Physical mixture and OA/mPEG-PCC-VE NPs were shown in Fig. 4. OA had intense diffraction peaks at 8.43, 12.99, 13.68, 15.36, 19.56 and 20.97°, suggesting that OA was present in crystalline form. However, there were no diffraction peaks of OA in OA/mPEG-PCC-VE NPs, suggesting almost complete drug amorphization.
3.3. **In vitro OA release profiles**

Drugs have to dissolve in gastric and/or intestinal fluids before they can permeate the membranes of the GI tract to reach the systemic circulation. Thus, increasing the dissolution rate of poor aqueous solubility drugs is an important issue in pharmaceutical research [18]. The in vitro dissolution profiles of OA and OA/mPEG-PCC-VE NPs in 900 ml aqueous solution containing 0.3% (w/v) SDS were shown in Fig. 5. OA/mPEG-PCC-VE NPs showed a superior dissolution profile and the time for 78% drug dissolution was 30 min, while the dissolution for physical mixture was only about 27% in the equal time. From the results, OA/mPEG-PCC-VE NPs dramatically improved the dissolution of OA. This could be explained by the special property of the carrier mPEG-PCC-VE which the ionized carboxyl groups favored the dissociation of the structure and the drug in cores would diffuse passively when it was in alkaline medium [23]. On the other hand, OA in the OA/mPEG-PCC-VE NPs was in an amorphous state, which enabled the drug to have higher internal energy and greater molecular motion, thus the dissolution of OA was improved. The modeling results established by DDSolve displayed in Table 1. Both the dissolution curves

![Fig. 3 – DSC thermograms of Blank (mPEG-PCC-VE), OA/mPEG-PCC-VE NPs, Physical mixture (OA+ mPEG-PCC-VE) and OA.](image1)

![Fig. 4 – XRPD patterns of Blank (mPEG-PCC-VE), OA/mPEG-PCC-VE NPs, Physical mixture (OA+ mPEG-PCC-VE) and OA.](image2)
of OA/mPEG-PCC-VE NPs and physical mixture conformed to the Weibull model ($r^2 = 0.9822, r^2 = 0.9880$).

3.4. **In situ SPIP study of OA/mPEG-PCC-VE NPs**

The in situ SPIP model in rats was used to evaluate the membrane permeability of OA/mPEG-PCC-VE NPs. As shown in Fig. 6, $K_a$ and $P_{app}$ of the OA/mPEG-PCC-VE NPs were significantly higher than those of OA solution in the whole intestinal segments, especially for colon and duodenum.

Since OA is a poorly water-soluble drug [18], a certain amount of sodium dodecyl sulfate was added in to improve the OA concentration of perfusion solution. According to our previous report [26], mPEG-PCC-VE as an orally nanosized carrier revealed enhanced the absorption in all segments of the intestine by confocal laser scanning microscope (CLSM), particularly colon exhibited the most absorption in a special mechanism, which was in an agreement with the $K_a$ and $P_{app}$ results in this study. What’s more, OA is the substrate of CYP3A4 [29] which is widely distributed especially at duodenum, so it can also be absorbed specially by duodenum after prepared into NPs.

3.5. **In vivo pharmacokinetic study**

The blood OA concentration versus time was shown in Fig. 7. The related pharmacokinetic parameters were displayed in Table 2. In comparison with the commercial tablets, the $C_{max}$

![Fig. 5 – Dissolution profiles of (A) OA/mPEG-PCC-VE NPs, (B) Physical mixture (OA+ mPEG-PCC-VE).](image)

![Fig. 6 – In situ SPIP, comparison of (A) $K_a$, (B) $P_{app}$ of OA/mPEG-PCC-VE NPs and OA solution (data are mean ± SD, n = 3).](image)

| Models       | Physicial Mixture | OA/mPEG-PCC-VE NPs |
|--------------|-------------------|--------------------|
| Zero order   | $y = 17.727 + 0.245x$ | $y = 68.410 + 0.131x$ |
|              | $r^2 = 0.8554$     | $r^2 = 0.5702$     |
| First order  | $y = 43.311 \times (1 - e^{-0.089x + 4.552})$ | $y = 79.673 \times (1 - e^{-0.089x + 11.018})$ |
|              | $r^2 = 0.9767$     | $r^2 = 0.9822$     |
| Higuchi      | $y = 7.722 + 3.451 \times x^{0.5}$ | $y = 62.239 + 1.980 \times x^{0.5}$ |
|              | $r^2 = 0.9508$     | $r^2 = 0.7323$     |
| Weibull      | $y = 585.620 \times \left(\frac{1}{1 + e^{-\frac{x - 2.906}{4.960}}}\right)$ | $y = 79.994 \times \left(1 - e^{-\frac{x - 8.112}{7.792}}\right)$ |
|              | $r^2 = 0.9880$     | $r^2 = 0.9840$     |
| Logistic     | $y = \frac{41.718}{1 + e^{-0.089(x - 10.332)}}$ | $y = 79.560 \times \left(1 - e^{-0.089(x - 11.796)}\right)$ |
|              | $r^2 = 0.9580$     | $r^2 = 0.9807$     |
of the group orally administrated the OA/mPEG-PCC-VE NPs was slightly improved. The $F_{rel}$ of OA/mPEG-PCC-VE NPs was 153%, demonstrating that mPEG-PCC-VE NPs developed in this study significantly enhanced intestinal absorption and oral bioavailability of OA.

Compared with sucrose ester-stabilized nanosuspensions of OA (SEOA) [15], the pharmacokinetic parameters of the prepared OA/mPEG-PCC-VE NPs are improved, especially the $t_{1/2}$. The $t_{1/2}$ of the sucrose ester-stabilized OA nanosuspensions is about 78 min while the $t_{1/2}$ of the OA/mPEG-PCC-VE NPs is 24.67 h, which is a dramatic improvement. Moreover, the $t_{max}$ and $F_{rel}$ of OA/mPEG-PCC-VE NPs are also improved compared with those of OA-phospholipid complex-hydroxyapatite (OPCH) [18]. The $t_{max}$ and $F_{rel}$ of OPCH are 0.46 h and 139.4%, while the $t_{max}$ and $F_{rel}$ of OA/mPEG-PCC-VE NPs are 0.729 h and 153.01%, respectively. We may attribute the enhanced pharmacokinetic parameters to the special property of the mPEG-PCC-VE. It could form the nanosized particles, which may prolong the residence time in the intestinal tract by physical absorption.

4. Conclusions

The OA/mPEG-PCC-VE NPs developed by mPEG-PCC-VE polymer as an effective drug delivery system were successfully prepared by solvent evaporation method. As compared to the physical mixture, the mPEG-PCC-VE NPs could significantly improve the dissolution of OA. The OA/mPEG-PCC-VE NPs can remarkably enhance the absorption of OA in intestinal tract, especially for duodenum and colon. After oral administration, the OA/mPEG-PCC-VE NPs showed higher $C_{max}$ and $AUC_{0-24h}$ in rat plasma. And the OA/mPEG-PCC-VE NPs exhibited a 1.53-fold increase in bioavailability when compared to the commercial available tablets. These results demonstrated that mPEG-PCC-VE NPs were a promising platform to facilitate the oral delivery of poorly water-soluble drugs.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Table 2 – Pharmacokinetic parameters after oral administration of OA commercial tablets (20 mg/kg) and OA/mPEG-PCC-VE NPs formulations at a dose equivalent to 12 mg/kg OA, respectively (mean ± SD, n = 5).

| Preparations          | Dosage (mg/kg) | $t_{1/2}$ (h) | $C_{max}$ (ng/ml) | $T_{max}$ (h) | $AUC_{0-24h}$ (ng/ml*h) | $F_{rel}$ (%) |
|-----------------------|----------------|---------------|-------------------|---------------|-------------------------|---------------|
| Commercial tablets    | 20             | 20.9 ± 15.3   | 40.15 ± 23.33     | 0.667 ± 0.56  | 321.98 ± 128.36         | 100           |
| OA/mPEG-PCC-VE NPs    | 12             | 24.67 ± 17.48 | 28.07 ± 13.89     | 0.729 ± 0.55  | 295.59 ± 112.7          | 153.01        |

Fig. 7 – Mean plasma concentration-time curves of OA in rats after oral administration of commercial tablets and OA/mPEG-PCC-VE NPs (data are mean ± SD, n = 5).
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