Synthesis, Characterization and *In Vitro* Evaluation of Self-Assembled poly(ethylene glycol)-glycyrrhetinic Acid Conjugates

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Abstract: Glycyrrhetinic acid (GA) is a commonly used drug for the chemotherapy of Chronic Hepatitis B, allergic dermatitis, inflammation, etc. But some problems such as poor water-solubility, low bioavailability and short plasma half-life, have limited its use. In the present study, PEGylation derivatives of GA derivatives were synthesized and characterized by FTIR, NMR, transmission electron microscopy, particle size analysis, etc. The PEG-GA conjugates having a critical micelle concentration of 0.081-0.73 mg/mL were used to form nano-sized micelles, with mean diameters of 120.86 ± 31.74 nm. The physico-chemical properties of the PEG–GA conjugate were evaluated including stability, cellular toxicity, and drug release profile. All the conjugates synthesized showed good stabilities in acidic and neutral solutions, while the stability in alkaline solution and the enzymatic hydrolysis rate, were significantly affected by the linkage between the GA and PEG chain. The results demonstrate that, by PEGylation of GA derivatives, the greatly increased water solubility and desirable self-assembly abilities of PEG-GA were obtained. The novel conjugates have potential medical applications for intravenously delivery of insoluble drug delivery.

Keywords: Glycyrrhetinic acid, *in vitro* evaluation, micelles, poly(etylene glycol), self assembly.

INTRODUCTION

18β-glycyrrhetinic acid (GA, 3β-hydroxyl-11-oxo-olean-12-ene-29-oic acid, 1) and glycyrrhizic acid (2) are the main active principles of the plant *Glycyrrhizae radix*, and GA is the hydrolysis active product of glycyrrhizic acid. The structures of GA and glycyrrhizic acid are displayed in Fig. (**1**). GA has been used as medicine to treat allergic and hepatic diseases [1]. GA suppresses the tumor promoting effect of 12-O-tetradecanoylphorbol-13-acetate (TPA) and 7,12-dimethylbenz[a]anthracene on skin tumor formation in mice [2], and has antitumor activities [3]. Recently, it has been shown that GA could induce apoptosis in human hepatoma, leukemia, and gastric cancer cells at high concentrations [4]. But, concomitantly, GA exhibited increased scarce stability, poor water solubility resulting in a decreased therapeutic index.

In our previous study, we constructed glycyrrhetinic acid-modified stealth cationic liposomes loaded with pDNA and found to transfect human hepatocellular carcinoma cell line HepG2 with high efficiency [5]. Recently, Lin *et al*. prepared chitosan nanoparticles modified with glycyrrhizic acid, and confirmed that modified nanoparticles preferentially accumulated in rat hepatocytes by a ligand-receptor interaction [6]. Mao *et al*. found that the cellular uptake of liposomes modified with glycyrrhetinic acid by rat hepatocytes was 3.3-fold higher than that of unmodified ones [7]. Tian *et al*. also reported a liver-targeted drug delivery carrier, composed of chitosan/poly(ethylene glycol) - glycyrrhetinic acid nanoparticles, prepared by an ionic gelation process, in which glycyrrhetinic acid acted as the targeting ligand [8].

Numerous strategies have been studied with the aim of improving the bioavailability of GA and to obtain a more rational therapeutic use of this active compound [9]. Among these strategies, the synthesis of GA derivatives has been widely used [9]. Unfortunately, choosing to synthesize new chemical entities from an original compound on one hand can increase the therapeutic efficacy but on the other hand can induce new and unexpected side-effects.

Recently, the possibility of synthesis a number of polyethylene glycol esters of GA and characterizing them to assess their chemical and enzymatic hydrolysis has been investigated [9d]. But this polymer–anticancer drug conjugates have been designed as potential dermal prodrugs, and the short hydrophilic chain of oligoethylene esters cannot provide self-assembly properties. However, so far, there has been no consensus regarding which strategy provides the optimal strategy outcome.

In this study, biodegradable GA-mPEG conjugate synthesized by Poly(ethylene glycol) monomethyl ether (mPEG), which is used most often since it is water soluble, biocompatible and nontoxic, facilitating its application for conjugation with paclitaxel, camptothecin and doxorubicin to improve their water solubility, plasma clearance and biodistribution, were investigated for their biodegradability as a means of reducing the cytotoxicity. As drug carriers, PEG can significantly change the biodistribution of the therapeutic agent, thus improving its pharmacokinetics(PK) and pharmacodynamics(PD), increasing their therapeutic effects and reducing their side effects[10].The mGA-mPEG

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was synthesized through succinic anhydride chain which was used as a bridge for the attachment of GA with polyethylene glycol monomethyl. Meanwhile, GA-mPEG was synthesized without succinic anhydride chain. The biodegradability was investigated by the hydrolysis of the ester groups in the synthesized GA and PEG using HPLC and the cytotoxicity was evaluated on the HEK293 cell line by the MTT assay.

In vitro release experiments in aqueous buffer demonstrate that both conjugates are stable in buffer of pH 7.4 and 1.2; mGA-mPEG show a good release of parent drug and GA-mPEG is stable in buffer of pH 10. The results showed that succinate chain was critical for attachment of GA with the polymer and the cleavage of ester linkage in simulated environment of blood. In addition, GA-mPEG and mGA-mPEG conjugate in nomal environment will be more stable than that in environment which was added in Porcine Pancreas Lipase. The conjugate of hydrophobic drug with the polymer might cause high bioavailability and low toxicity.

MATERIALS AND METHOD

Materials

18β-glycyrrhetinic acid (GA, purity >98% by HPLC) was purchased from Fujie Pharmaceutical Co., Ltd. (Xi'an, China). 4-Dimethyl amino pyridine (DMAP), poly(ethylene glycol) mono-methyl ether (mPEG, Mr = 2000), succinic anhydride, EDC were purchased from Sigma. All the other reagents and solvents used in this work were of the analytical grade and were used without further purification except chloroform. ^{1}H NMR analysis was performed by the INOVA VARIAN 400MHz spectrometer using $CDCl_3$ or DMSO-*d6* as a solvent at room temperature. The infrared spectra were also measured using an IR spectrometer (Nicolet 5DX FTIR). The samples were scanned from 400 to 4000 cm^{-1}.

Synthesis of Glycyrrhetinic Acid Methyl Ester (mGA, 1a)

The synthesis process of GA–PEG conjugates was displayed in Scheme **1**. Synthesis of glycyrrhetinic acid methyl ester (mGA, 1a) and glycyrrhetinic acid methyl ester 3-O-hemisuccinate ester (mGA –suc, 1b) were carried out according to the literature [5]**.** Briefly, to a solution of 1 (15.01 g, 31.9 mmol) in methanol (500 mL) was added p-toluenesulfonic acid (3.44 g, 20mmol). The mixture was refluxed for 72 h, concentrated *in vacuo*, poured into water, extracted with dichloromethane. The organic phase was washed with brine, dried over magnesium sulfate and concentrated to yield white solid. White solid (10.1 g, 72%) were obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ5.77 (s, 1H, 12-H), 3.70 (s,3H, $-OCH_3$), 3.23 (m, 1H, 3a-H), 2.80 (dt, J=13.6,4.5 Hz, 1H, 1β-H), 2.37 (s, 1H, 9a-H), 1.37 (s, 3H, CH_3),1.15 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 1.13 (s, 3H, CH_3),1.01 (s, 3H, CH_3), 0.81 (s, 6H, 2*CH_3); ESI-MS m/z:485.4 (100%, $M+H^+$), 507.4 ($M+Na^+$).

Synthesis of Glycyrrhetinic Acid Methyl Ester 3-O-hemisuccinate ester (mGA –suc, 1b)

Glycyrrhetinic acid methyl ester 3-O-hemisuccinate (1b) was prepared by dissolving 2.01g (4.16 mmol) of 1a in 80 mL of pyridine. Then 0.25g (2.05mmol) of DMAP and 2.08 g (20.8 mmol) of succinic anhydride were added. The mixture was stirred for 48 h at 100 ˚C, then the solution was then concentrated under vacuum, and the resulting precipitate was extracted with dichloromethane, then DMAP was washed off by 1mol/L HCl. The combined organic layer were dried on anhydrous Na_2SO_4 and concentrated. The crude product was purified on a silica gel chromatography column. White solid (1.933 g, 80%) were obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ5.77 (s, 1H, 12-H), 3.70 (s,3H, $-OCH_3$), 3.23 (m, 1H, 3a-H), 2.80 (dt, J=13.6,4.5 Hz, 1H, 1β-H), 2.5-2.7 (brs, 4H, suc, $COCH_2$*2) , 2.37 (s, 1H, 9a-H), 1.37 (s, 3H, CH_3),1.15 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 1.13 (s, 3H, CH_3),1.01 (s, 3H, CH_3), 0.81 (s, 6H, 2*CH_3); ESI-MS m/z:585.4 (100%, $M+H^+$)).

Synthesis of GA-mPEG Conjugate (GA-mPEG, 1c)

To a solution of GA (4.00 g, 8.50mmol) in chloroform (500mL) was added 1.04g (8.50mmol) of DMAP and 3.58 g (18.70mmol) of EDC, then 8.50g (4.25mmol) $mPEG_{2000}$ were added. The mixture was refluxed for 24h at 35 ˚C, then the solution was then concentrated under vacuum, and the resulting precipitate was extracted with dichloromethane, then DMAP was filtered off by 1mol/L HCl. The combined organic layer were dried on anhydrous Na_2SO_4 and concentrated. The GA-mPEG (1c) were purified on a silica-gel column, eluting with a mixture of DCM- methanol (100:1), white solid (4g, 69%) was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ5.77 (s, 1H, 12-H), 3.64-3.76 (m,90H, MPEG , CH_2*45), 3.23 (m, 1H, 3α-H), 2.37 (s, 1H, 9α-H), 1.37 (s, 3H, CH_3),1.15 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 1.13 (s, 3H, CH_3),1.01 (s, 3H, CH_3), 0.81 (s, 6H, 2*CH_3); IR (film) υ: 2886, 2696, 2239, 1727, 1658, 1146, 1113.

Synthesis of mGA-mPEG Conjugate (mGA-mPEG, 1d)

The mGA-mPEG conjugate (1d) prepared from dissolving 2.00g (3.44 mmol) of 1b in 50 mL of dichloromethane. Then 0.42 g (3.44mmol) of DMAP and 0.72 g (3.78mmol) of EDC and 6.87g (3.44 mmol) $mPEG_{2000}$ were added. The mixture was stirred for 12 h at room temperature, then DMAP and EDC was washed off by 1N HCl. The organic phases were collected, dried by Na_2SO_4 and the solvent was evaporated *in vacuo*. The products (1d) were purified on a silica-gel column, eluting with a mixture of DCM-methanol (60:1), White solid (2.4 g, 27%) were obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ5.77 (s, 1H, 12-H), 3.64 (m,90H, MPEG , CH_2*45), 3.23 (m, 1H, 3α-H),2.5-2.7 (brs, 4H, suc, $COCH_2$*2) ,2.37 (s, 1H, 9α-H), 1.37 (s, 3H, CH_3),1.15 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 1.13 (s, 3H, CH_3),1.01 (s, 3H, CH_3), 0.81 (s, 6H, 2*CH_3); IR (film) υ: 2741, 2696, 1969, 1732, 1659,1147.

In Vitro Degrade Experiments

To evaluate the chemical and enzymatic stability of esters 1c and 1d, *in vitro* release kinetics of GA or mGA from above conjugation were carried out at 37 ± 1℃. Briefly, 10 mg of conjugation(1c-1d) without free drugs was put in kimax, which contained 40 mL pH 7.4 phosphate buffer solution or pH 10 buffer solution ($NaCO_3$/$NaHCO_3$)

or pH 1.2 HCl solution to maintain the stable condition and shaken in a constant temperature shaker at 100 rpm. At regular time intervals, 0.3mL of the release medium was removed and replaced with 0.3 mL of the fresh release medium. The samples were subjected to HPLC assay.

Enzymatic hydrolysis of esters was determined as previously reported [11]. Porcine pancreas lipase was diluted 300 fold with phosphate buffer pH 7.4 before use. A 37mL volume of phosphate buffer solution was added in 3 mL of phosphate buffer which contained enzyme, a constant temperature at 37 ˚C, and then conjugates were added respectively. The formation of the GA or mGA in the solution was monitored by the HPLC reported below.

HPLC Analysis

The HPLC apparatus consisted of a Shimadzu LC-20AD equipped with a Shimadzu SPD-20A UV detector. Chromatography was performed using an ODS Hypersil column (particle size, 5 μm; 25 cm × 4.6 mm i.d; Thermohypersil, Bellefonte, PA, USA). The mobile phase used was 1% acetic acid aqueous solution/methanol (7:93) and the flow rate was 1.0 mL/min. Each sample was filtered before injection with a Millex HV13 filter(Waters-Millipore Corporation, Milford, MA, USA) and a volume of 20 μL was injected into the HPLC apparatus. The wavelength of the UV detector was set to 254 nm.

Preparation of Micelles

Micelles were prepared by film formation method [12]. GA-mPEG or mGA-mPEG(10mg) was weighted into a round-bottomed flask and dissolved in 2.5 mL chloroform. The solvent was evaporated under reduced pressure at 37 ˚C for 30min. The resulting film was hydrated and redispersed with 2.0 mL doubly distilled water at 37 ˚C for 15min. Micelles were stored at 4 ˚C until use.

Determination of Ritical Micelle Concentration (CMC)

Fluorescence spectra were recorded on a Shimadzu RF-5301 fluorescence spectrophotometer. Pyrene was used as a fluorescence probe to analyze the GA-mPEG or mGA-mPEG in doubly distilled water. Samples for fluorescence measurement were prepared according to the literature [13], and the concentration of the aqueous solutions ranged from 1.0×10^{-6} to 10 mg/mL. The pyrene concentration in the solution was chosen to be 6.0×10^{-7} M. For the measurement of pyrene excitation spectra, the slit widths for both excitation and emission sides were maintained at 3 nm, and an emission wavelength of 374 nm was used. The intensity ratios of I_{337}/I_{333} were plotted as a function of logarithm of the GA-mPEG or mGA-mPEG concentration. The CMC value was taken from the intersection of the tangent to the curve at the inflection with the horizontal tangent through the points at low concentrations [14].

Particle Size

The size distribution spectra of mGA-mPEG micelles were determined using a Zetasizer Nano ZS90 (Malvern Instruments, Ltd., UK). The samples were suitably diluted with ultrapurified water. Experiments were performed in triplicate.

Transmission Electron Microscopy

The morphology of mGA-mPEG micelles was observed by TEM (H-600, Hitachi, Japan). Before analysis, the samples were diluted 1:5 and negatively stained with 2% (w/v) phosphotungstic acid for 30s, and then placed on copper grids precoated with a thin film of poly(vinyl formaldehyde) for observation.

Cytotoxicity Experiment

Cytotoxicity of various blank micelles were determined by MTT assay on HEK293 cell lines as described before [5]. Cells were seeded on 96-well plates (Corning Incorporated, NY, USA) at a density of 3×10^{3} cells per well. Following attachment for 24 h, cells were treated by various blank cationic liposomes with different concentrations. These treatment agents were incubated with cells for an additional 48 h. Then 20 μl of MTT stock solution (5 mg/mL in saline)

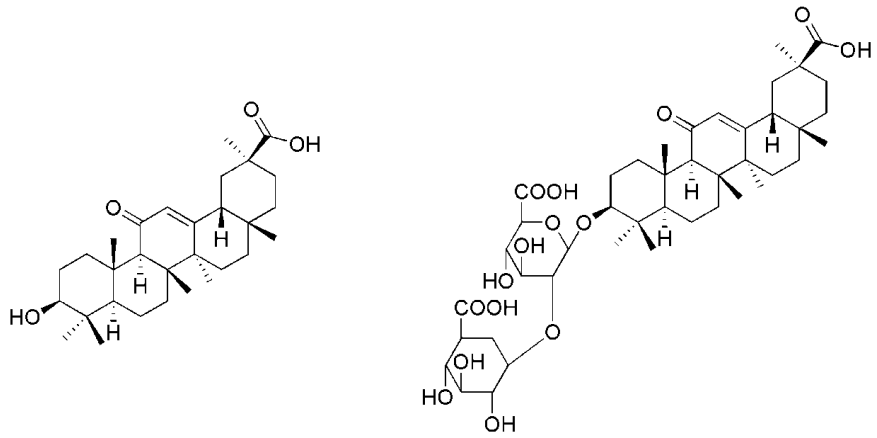


Fig. (1). Chemical structures of glycyrrhetinic acid (GA) and glycyrrhizic acid (GL).

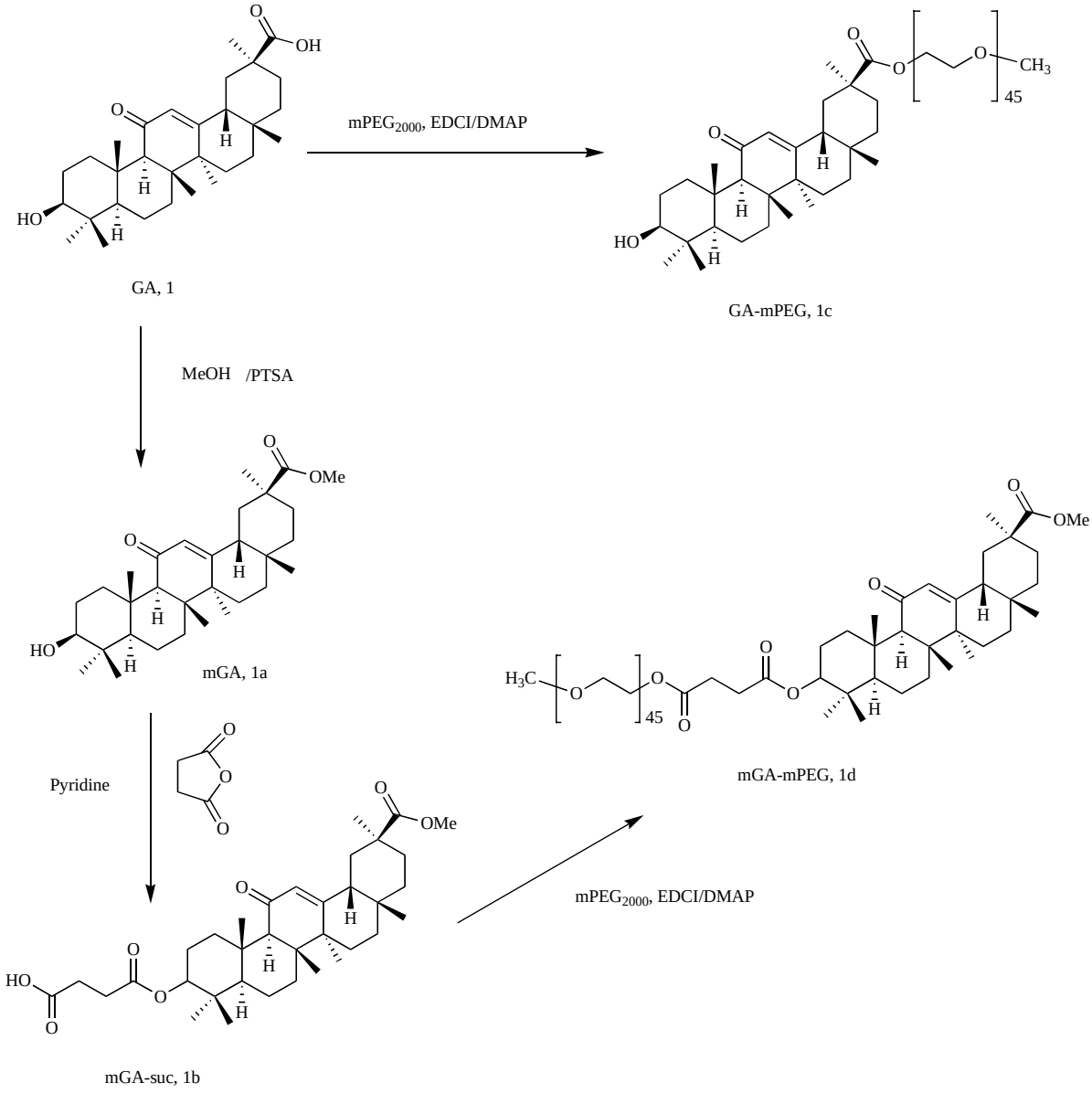
was added to each well and the cells were further incubated at 37 °C for 4 h. Finally, the culture medium was removed by aspiration and then 150 µl of dimethyl sulfoxide (DMSO) was added to each well to dissolve formazan crystals. The absorbance of each well was read at 490 nm on a Bio-Tek µQuant Universal Microplate Spectrophotometer (Bio-Tek Instruments Inc, Winooski, VT, USA). The relative cell viability compared to control was calculated as A sample/A control ×100%. All the experiments were repeated in triplicates.

RESULTS AND DISCUSSION

Preparation of Drugs-Polymer Conjugates

The glycyrrhetinic acid-mPEG conjugate was successfully prepared by using the synthetic route as shown in Scheme **1**. The method of choice for coupling bioactive components to the PEG backbone is mainly by esterification. This type of esterification can be divided into two main approaches: (a) activation of the hydroxy end group through transformation into a good leaving group and subsequent attack by the carboxylate component, such as formation of PEG-isourea and PEG-tosylate; and (b) activation of the carboxy component and subsequent attack by PEG hydroxy end groups, such as the direct coupling by carbodiimide. Particularly, it was demonstrated that DMAP catalyzed attachment of carbodiimide-activated glycyrrhetinic acid proceeds good yield with PEG under very mild conditions. Therefore, we employed this EDC/DMAP method in the synthesis of both PEG-amino acid derivatives and the final glycyrrhetinic acid-mPEG conjugates.

The FT-IR spectra of mPEG, GA-mPEG and mGA-mPEG were showed in Fig. (**2**). The broad band at around 3480 cm^{-1} attributed to the inter-and intra-molecular hydrogen bonding of –OH stretching vibration of 3-OH of glycyrrhetinic acid in GA-mPEG, because the corresponding



Scheme 1. Synthesis route of GA-mPEG and mGA-mPEG.

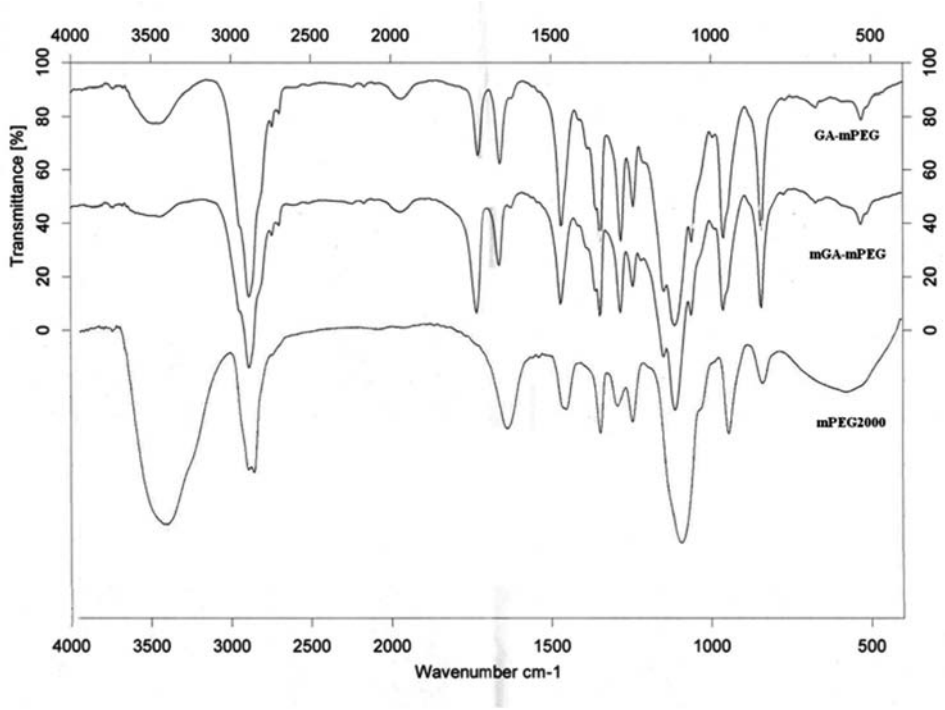


Fig. (2). FT-IR spectra of mPEG, GA-mPEG and mGA-mPEG.

hydroxyl group in mGA-mPEG had been esterification, the band around 3480 cm^{-1} had disappeared. The strong peaks around 2880cm^{-1} were assigned to the -CH_2- which was brought by mPEG. The sharp peak at 1112 cm^{-1} was assigned to the C-O group which was brought by the mPEG. Another small peak at 1730 cm^{-1} was belong to carbonyl group of ester linkage between glycyrrhetinic acid and mPEG. Other prominent peaks at 1340 cm^{-1} and 1460 cm^{-1} were assigned to the asymmetrical and symmetrical bending vibrations of methyl, methylene groups which were the introduction of long PEG chain.

Fig. (**3**) shows the ^{1}H NMR spectra of GA-mPEG, mGA-mPEG and mGA-suc in $CDCl_3$ or d_6-DMSO. The single peak at δ 5.69 and 5.60 (a) was attributed to the protons of olefinic bond (–(C=O)-CH=C-) in GA. The peaks at δ 2.58-

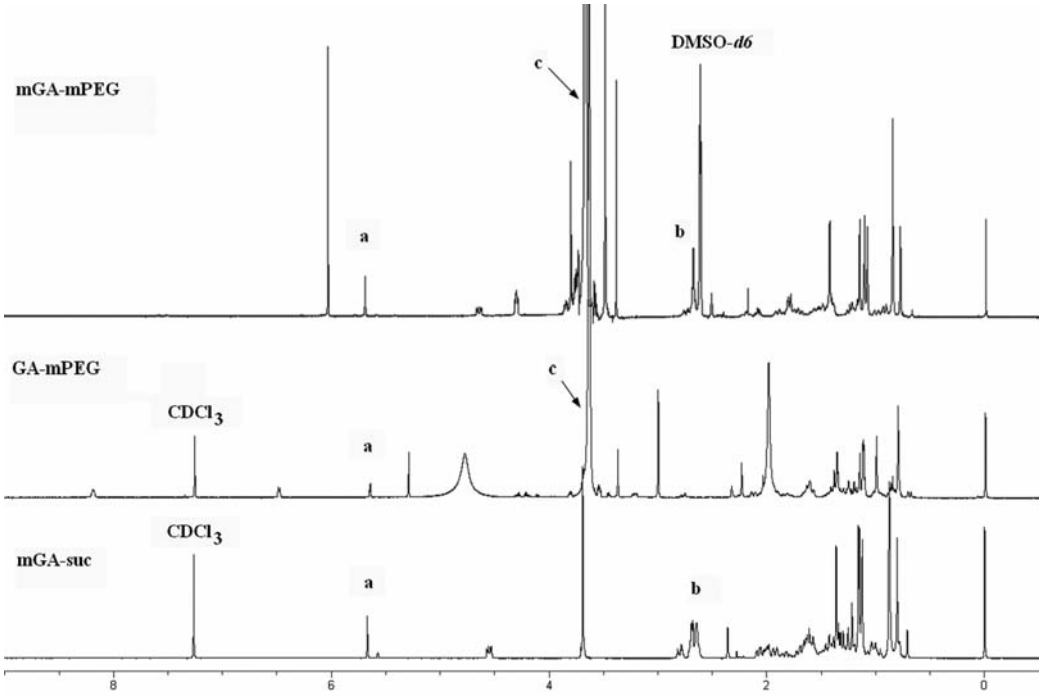


Fig. (3). ^{1}H NMR spectra of mGA-suc, GA-mPEG in $CDCl_3$ and mGA-mPEG in DMSO-d_6.

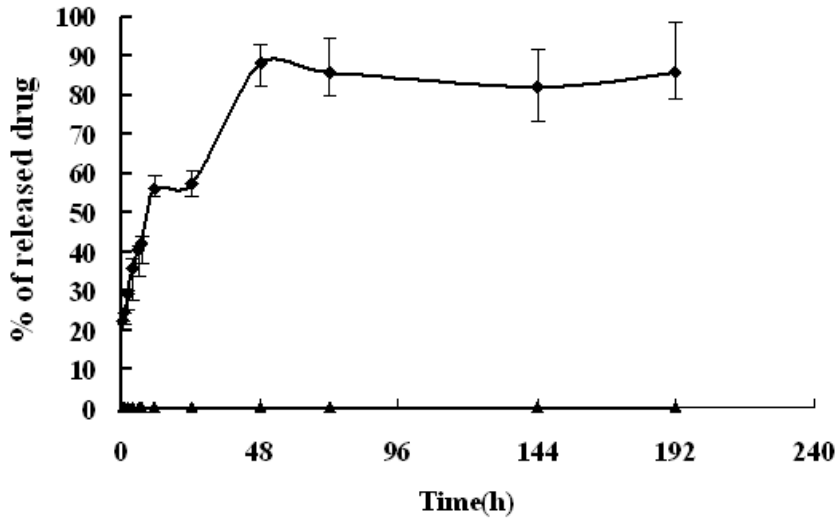


Fig. (4). Realease of GA or mGA from corresponding micells in alkline solution.

◆Release of mGA from mGA-mPEG in pH10 ($NaCO_3$/$NaHCO_3$)phosphate buffer at 37°C, ▲release of GA from GA-mPEG in pH 10 ($NaCO_3$/$NaHCO_3$)phosphate buffer at 37°C.

2.66 (b) came from the protons of succinate linkage (–(C=O)-CH2-CH2-(C=O)-). The peaks at δ 3.52-3.76 (c) were attributed to the protons from the glycol unit (-O--CH2-CH2-O-) in PEG chain. Thus, GA-mPEG and mGA-mPEG conjugates have been successfully synthesized.

Hydrolysis of Conjugates in Different pH

The hydrolysis rate of mGA-mPEG in buffer solutions in different pH is the following: at pH 1.2, after 192h, there is no mGA-mPEG hydrolyzed; at pH 7.4(phosphate buffer solution), the result is as similar as pH 1.2; at pH 10, after 48 h, is hydrolyzed by 87.8% and keep the stable level until 192h. The mGA-mPEG release in buffer solutions at three different pH shows alkaline environment was critical for the cleavage of ester linkage.

The results showed that succinic anhydride chain was critical for the cleavage of ester linkage in the alkaline environment and also demonstrated that the conjugation without succinic anhydride chain (1c) is more stable than conjugation (1d). Moreover, the figure showed the release ratio of mGA and GA was probably due to steric hindrance and the synthesis approach of drug-polymer conjugate. Above all, the existance of succinic anhydride chain is the main force in hydrolysis of GA oligoethylene conjugate.

Enzymatic Hydrolysis of Conjugates

In the fisrt 24 hours, about 86% of drug released from mGA-mPEG conjugates, but only 32% of drugs released from GA-mPEG in the same time. In contrast to the release profile of mGA-mPEG in pH 10 (in Fig. **4**, only 50% of drugs released from mGA-mPEG without enzyme after 24 h), we speculate that mGA-mPEG might be more effective than the other conjugation in the treatment of drug resistant cancers and Porcine Pancreas Lipase might improve the drug release from the polymer.

Preparation and Characterization of Self-Assembly Micelles

As shown as Fig. (**6**), at a low concentration range, a negligible change of intensity ratio (I_{337}/I_{333}) was monitored. As the GA-mPEG or mGA-mPEG concentration increased, the intensity ratio exhibited a substantial increase at a certain concentration, suggesting that pyrene molecules were incorporated into the hydrophobic core region upon micelle formation. The critical micelle concentrations were therefore determined from the crossover point at the low concentration range [13]. As well-known, CMC value of pharmaceutical micelle is an important parameter and expected to be in a low millimolar region or even lower [15] (Torchilin **2007**).Similarly to the CMC values of PEG_{2000}-PE [16] (Wang *et al*., **2010**) $mPEG_{2300}$-DSPE [17] (Han *et al.* **2009**) determined by pryene fluorescence probe and $mPEG_{2200}$-Cholesterol [18] Ishiwata *et al*., **1995**) determined by surface tension, the CMC values of mGA-mPEG and GA-mPEG were shown to be as low as 3.17×10^{-5} M and 2.96×10^{-4} M, respectively. These results indicated that the mGA-mPEG and GA-mPEG micelles had good stability and they also had the properties to retain the integrity even upon strong dilution in the body. In our study, the mean size of mGA-mPEG micelles were 120.86 ± 31.74 nm (mean±SD; n=3) with a distribution from 18.72 nm to 42.67nm (PDI=0.24) (Fig. **7A**), further transmit electronic microscopy analyses confirmed that the micelles were spheroids with regular shape and a size distribution of 80-160nm (Fig. **7B**).

In Vitro Cytotoxicity

Although, mPEG has been proved to be non-toxic, tissue-compatible, its conjugates with glycyrrhetinic acid should be carefully checked before it is used as biomaterials. Fig. (**8**) shows the dependence of the concentration of mPEG, GA, mGA-suc, GA-mPEG, mGA-mPEG and glycine on the relative cell availability of cell culture (HEK293). From

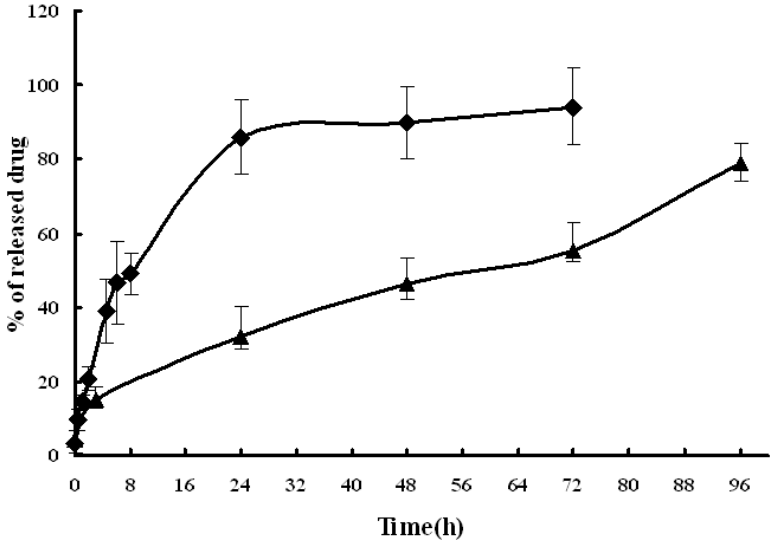


Fig. (5). Realease of GA or mGA from corresponding micells by enzyme catalysis.

◆*In vitro* release profile of mGA from mGA-mPEG in pH 7.4 phosphate buffer which contained Porcine Pancreas Lipase at 37°C, ▲release of GA from GA-mPEG in the same environment.

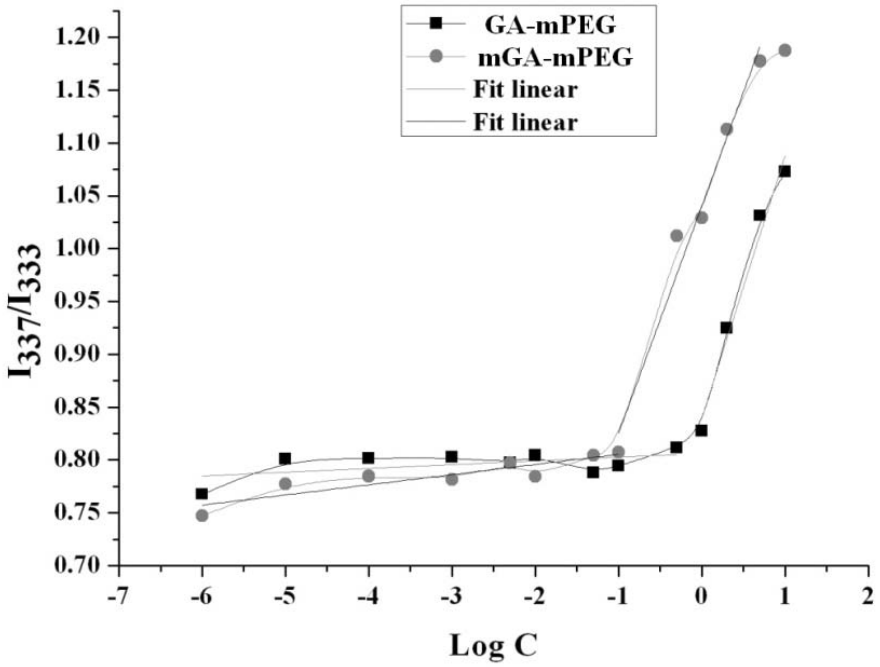


Fig. (6). The CMC(critical micelle concentration) of GA-mPEG and mGA-mPEG.

Fig. (**8**), we could find that there was not obvious cytotoxicity against HEK293 in 48 h culture as GA-mPEG and mGA-mPEG micelles concentration were ranging from 0 to 1.0 mg/mL. Meanwhile, the mPEG and mGA-suc were employed to investigate their cytotoxicity. According to Fig. (**8**), we could find that the mPEG was low-toxic as polymer concentration was ranging from 0.03 to 1.0 mg/mL, and the mGA-suc demostrated slightly growth inhibition activity as polymer concentration was more than 0.2mg/mL. These findings demonstrated that both GA-mPEG and mGA-mPEG micelles were low toxic, and cell-compatible. We will develop these micelles as the drug carrier for the hydrophobic drugs (such as hydrophobic anticancer drug) in the further research.

CONCLUSION

Drug-polymer conjugation was prepared and its physico-chemical properties, hydrolysis kinetics and cytotoxicity were investigated. The results showed that succinate chain was critical for the attachment of GA with the polymer and the cleavage of ester linkage in simulated the environment of blood. It was found that Porcine Pancreas Lipase might accelerate the drug release from the polymer. The conjugation of glycyrrhetinic acid the mPEG might cause high bioavailability. The cytotoxicity was evaluated on the HEK293 cell line by the MTT assay show that the conjugate of glycyrrhetinic acid with the polymer cause low toxicity. Although, further investigation on these Glycyrrhetinic

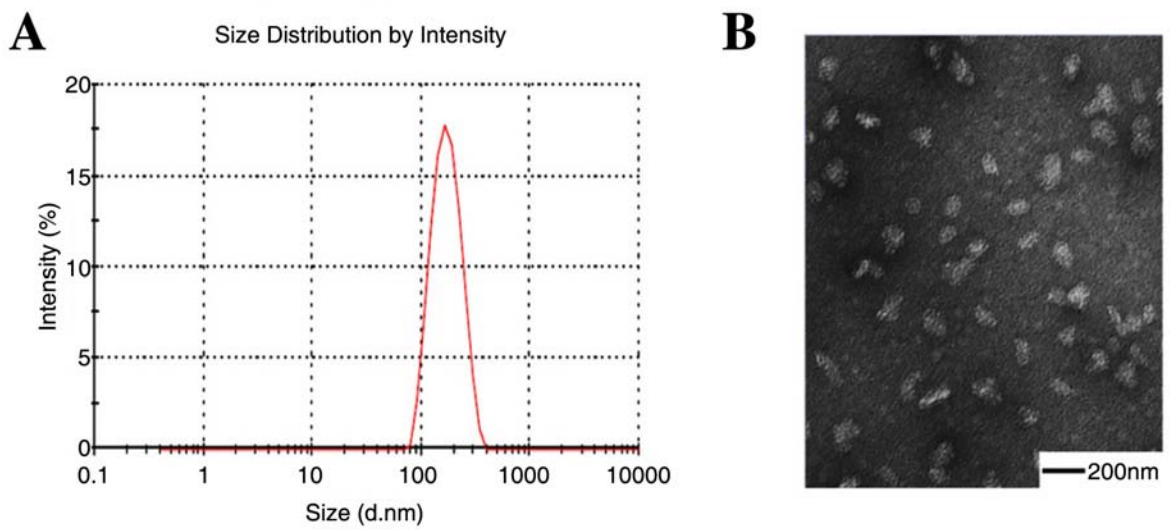


Fig. (7). Size distribution(A) and a typical TEM image of mGA-mPEG micelles.

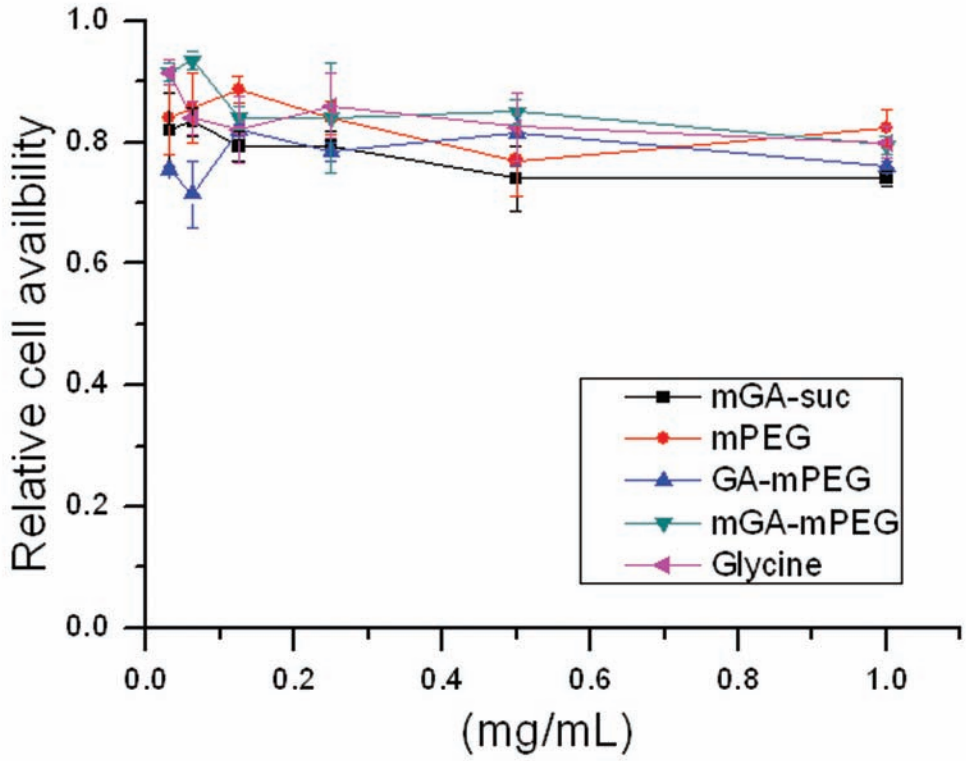


Fig. (8). Cytotoxicity of the mPEG, mGA-suc, GA-mPEG, mGA-mPEG and glycine on HEK293 cell lines. The percentage of viable cells was quantified using the methylthiazoletetrazolium method. Mean values and 95% confidence intervals derived from three independent experiments are shown.

acid- poly(ethylene glycol) conjuates are required, the findings of our study represent an important step in advancing the use of GA-mPEG and mGA-mPEG micelles as a potent strategy to novel drug delivery system.

CONFLICT OF INTEREST

Declared none.

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