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Synthesis of poly(ethylene glycol)–metaxalone conjugates and study of its controlled release in vitro

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Abstract

Metaxalone (Met), a drug for treatment of pain and stiffness due to muscular injuries, was covalently linked to poly(ethylene glycols) (PEG) via a chloroacetyl chloride spacer. The average weight molecular weights used for PEG are 4000, 6000 and 10,000, respectively, and the procedure of chemical modification for PEGs was conducted by a two-step protocol: (1) synthesis of *N*-chloroacetyl-metaxalone; (2) synthesis of PEG_{4000}–Met, PEG_{6000}–Met and PEG_{10000}–Met. The controlled drug release studies were performed in buffer solutions with pH values equal to 1.1, 7.4 and 10.0. The results demonstrate that, in the same condition, the rate of hydrolysis for PEG_{10000}–Met is the slowest among three prodrugs, and more amount of metaxalone can be detected releasing from prodrug matrices at the presence of α-chymotrypsin in a buffer solution with pH 8.0. It was also found that these novel prodrugs can effectively improve the metaxalone's pharmacokinetics, and furthermore can markedly increase its half-life period.

Keywords: Metaxalone; Poly(ethylene glycol); Polymer prodrug; Controlled release; *N*-Chloroacetyl-metaxalone

1. Introduction

Metaxalone, also known as Skelaxin with its chemical name of 5-[(3,5-dimethylphenoxy)methyl]-2-oxazolidinone, is a muscle relaxant, used to treat pain and stiffness of muscle injuries, including strains, sprains and muscle spasms. It works by blocking nerve impulses (or pain sensations) that are sent to the brain. It may be related to its sedative structure (Peter and Jason, 2004). Metaxalone can be metabolized by liver and excreted via urine. Its half-life period is 2–3 h (Cacace et al., 2004), but the low solubility in water restricts its widely clinical applications. The side effects of metaxalone for human being are presented with drowsiness, dizziness, blurred and low blood pressure (Harden and Argoff, 2000). Therefore, it is desirable to develop a delivery system for metaxalone that can effectively control its pharmacokinetics and enhance its therapeutic result and at the same time, reduce its adverse effects. To date, many novel approaches have been proposed to overcome these restrictions for effective utilization of small molecular drugs (Ulbrich et al., 2004).

Water-soluble polymers are model compounds for studying the metabolism of macromolecules in cells, and also are suitable for being used as drug delivery vectors. Polymer drug delivery systems can often compensate some shortcomings of small molecular drugs such as side effects, limited water solubility, poor biocompatibility, bio-stability, immunogenicity and so on (Jensen et al., 2003).

The preparations of polymeric drug delivery systems, in which a small molecular drug is covalently bonded to the backbone of polymers that is of hydrolysable in the body fluid, are currently recognized as an effective way to prolong the drug's pharmacological activity (Christic and Granger, 2003; Ulbrich and Šubr, 2004). The mechanism of this technology can make small molecular drugs gradually free from polymer matrixes if they are subjected to hydrolysis of body fluid. Among these water-soluble polymers, poly(ethylene glycols) (PEG) appears to be particularly convenient as oligomeric matrices, since they are available in a wide range of desired molecular weights, and possessing a well defined macromolecular structure. PEG is also known to be non-toxic, non-antigenic, biocompatible and

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soluble in both water and most organic solvents. PEG can transfer its properties to another molecule relatively easily when it is covalently bonded to this molecule. In this way, it could make the toxic molecules become non-toxic, or hydrophobic molecules become water soluble. Besides, coupling of a biological molecule into PEG's macromolecules usually can still keep this molecule's biological activity. Because of these unique properties of PEG, it is very suitable for being used as a drug carrier, and it can also be rapidly eliminated from the human body (Bersani et al., 2005; Subbu et al., 2005).

Considering all these general properties and advantages of PEG, in this research work, we selected PEG as a polymer drug carrier and metaxalone as a small molecular drug which was covalently linked to PEG for preparing PEG–Met prodrug. By using PEGs with different molecular weights, several polymer prodrugs were synthesized, and their detailed molecular structures were characterized via NMR and FTIR techniques. The products obtained showed markedly improved solubility and half-life period. Furthermore, study in vitro on drug release from these prodrugs in various media demonstrated that controlled release effect for Met has been achieved significantly.

2. Materials and methods

2.1. Materials

Metaxalone (99.9%, w/w, analyzed by GC) was synthesized according to the method reported in the literature (Lunsfurd et al., 1962). PEG with average molecular weight of about 4000, 6000 and 10,000, were of analytic grade made in Tian-Tai Fine Chemical Ltd. (China); α-chymotrypsin was purchased from Kaiyau Medicine Co. (Beijing, China). Dialtic bag (cut-off molecular weight 3500) was obtained from Huamei Biochemical Co. (Beijing, China). Chloroacetyl chloride was of a chemical grade from Guoyao Chemical Co. (Shan-Xi, China); triethylamine, toluene, petroleum ether, dimethyl sulfoxide and other chemicals were of analytical grade and were made in China; they were used as received without further purification.

2.2. Analytical methods

^{1}H NMR measurements were conducted on Varian INOVA-400 spectrometer at room temperature with $CDCl_3$ as a solvent. Infrared spectroscopy experiments were preformed on a Specode 75 model (Carl Zeiss, Jena, Germany) using KBr as the sample holder. Elemental analyses were carried out on a Vario EL III instrument. Ultraviolet–visible spectra were recorded on a UV-1700 spectrophotometer (Beijing, China). DSC (MDSC 2910, TA Instruments) were used to determine the thermal behaviors of metaxalone and prodrugs. The scan rate was set to 20 °C/min within the temperature range of 30–300 °C.

2.3. Synthesis of N-chloroacetyl-metaxalone

In a three-neck round bottomed flask equipped with a magnetic stirring bar and reflux condenser (Mocanu et al., 1996), 0.5 g of metaxalone and 0.3 g of triethylamine were dissolved in 15 ml of toluene together with 0.41 g chloroacetyl chloride were dropwise added under stirring. The system was maintained at 50 °C for 30 min, and then, to be heated to reflux for 3 h. After completing the reaction, the system was cooled down to room temperature. The excessive chloroacetyl chloride was washed off by water, the organic layer was separated. The solvent was removed under reduced pressure. The white product was precipitated and filtered. The product was recrystallized twice with toluene. Yield: 0.63 g (92.6%, w/w). Rf = 0.72 (1:10 ethyl acetate: dichloromethane).

Anal. calc. for $C_{14}H_{15}NO_4Cl$: C, 56.32; N, 4.85; H, 5.54. MW. 296.7. Found: C, 56.67; N, 4.72; H, 5.10%.

IR (KBr): 1774 (O=C, ester), 1713 (O=C amide), 1232 (C–O–C=O, ester), 1072 (Ar–O–C, ether) cm^{-1}. ^{1}H NMR (δ, ppm): 2.29 [s, 6H, $(CH3)_2$–Ar], 4.12 (m, 2H, CH_2–N), 4.22 (m, 2H, Ar–O–CH_2) 4.76 (s, 2H, CH_2–Cl), 4.98 (m, 1H, CH_2–CH–O), 6.51 (s, 2H, H–Ar), 6.66 (s, 1H, H–Ar). Melting point: 152–153 °C.

2.4. Synthesis of alcoholic sodium of poly(ethylene glycol)

In a 100 ml three-neck round bottomed flask equipped with a magnetic stirring bar and reflux condenser, 2.0 g of PEG (PEG_{4000}) dissolved in 10 ml of toluene plus 0.03 g of Na were added under stirring and heated to 100 °C for 4 h. And then, the system was cooled down to room temperature, the excessive Na was filtered off, the product (**1**) was kept intactly for next step reaction.

2.5. Synthesis of PEG_{4000}–Met conjugate

In a 100 ml three-neck round bottomed flask equipped with a magnetic stirring bar and reflux condenser containing product (**1**) above, 0.34 g of *N*-chloroacetyl-metaxalone was added under stirring and heated to 50 °C for 15 h, the solvent was evaporated under vacuum, the system was cooled down to room temperature, and the product was obtained by precipitating it into 20 ml of distilled petroleum ether, and then filtered. The product was washed three times with petroleum ether, and finally, a white crystal was gotten and dried under vacuum.

The pure PEG_{4000}–Met conjugate with the yield of 87.3% was obtained. The fact that there was no free drug of Met existing in polymer prodrug was confirmed by thin layer chromatography and DSC measurements. Standard curve was made by pure metaxalone in water solution. The content of metaxalone in the conjugate was calculated by comparing the slopes of the standard curve of metaxalone at 271.0 nm wavelength, on the basis of the release metaxalone in alkaline media (Chen et al., 2000) after 1 h at 60 °C. It was confirmed that metaxalone content in the conjugate was 9.8%.

IR (cm^{-1}): 2906 (–CH_2–CH_2–, PEG), 1747 (O=C, ester), 1639 (O=C, amide), 1597 (Ar), 1105 (C–O–C, ether), 700 (Ar-1,3,5 substitute).

^{1}H NMR (δ, ppm): 3.6 (s, 2H, C$\underline{H_2}$–O–C$\underline{H_2}$), 2.29 (s, 4H, Ar–C$\underline{H_3}$), 6.64 (s, 0.48H, Ar–$\underline{H}$), 6.54 (s, 1H, Ar–$\underline{H}$), 4.12 (m, 3H, C$\underline{H_2}$–N), 4.35 (m, 1H, C$\underline{H}$–O), 4.98 (m, 1H, O–C$\underline{H_2}$–CH).

The same procedure was used to prepare PEG_{6000}–Met as mentioned above. In this case, 3.0 g of PEG_{6000} was dissolved in 10 ml of toluene, and 0.03 g of Na, 0.34 g of *N*-chloroacetyl-metaxalone was used. Yield: 88.5%. Metaxalone content in the conjugate was about 7.6%.

IR (cm^{-1}): 2889 (CH_2–CH_2, stretch, PEG), 1749 (C=O, stretch, ester), 1639 (C=O, stretch, amide), 1599 (Ar), 1113 (C–O–C, stretch, ether), 694 (Ar-1,3,5 substitute).

The same procedure was used to prepare PEG_{10000}–Met. In this case 5.0 g of PEG_{10000} was dissolved in 10 ml of toluene, and 0.03 g of Na, 0.34 g of *N*-chloroacetyl-metaxalone was used. Yield: 83.6%. Metaxalone content in the conjugate was about 4.2%.

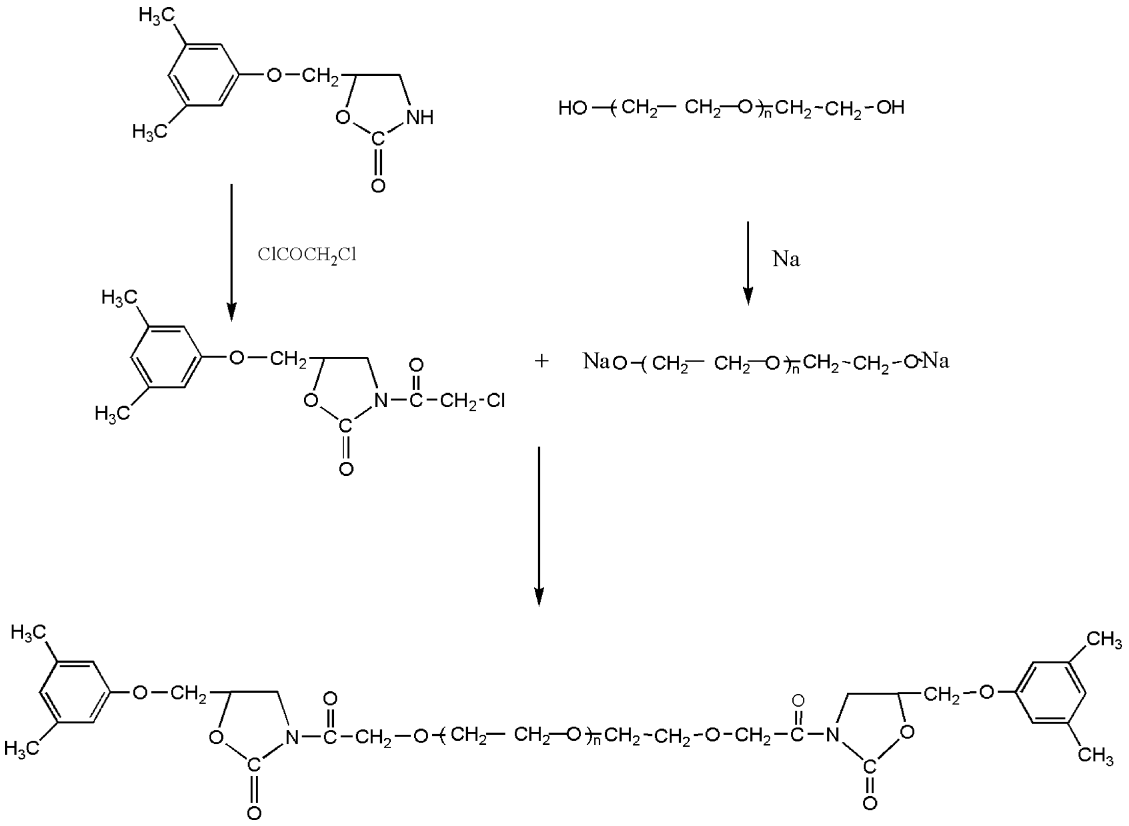
IR (cm^{-1}): 1747 (C=O, stretch, ester), 1712 (C=O, stretch, amide), 1590 (Ar), 1113 (C–O–C, stretch, ether), 688 (Ar-1,3,5 substitute).

2.6. Drug release tests via hydrolysis

The drug release experiments via hydrolysis were carried out at pH 1.1 (0.002 M glycine–0.002 M KCl adjusted to pH 1.1with HCl), pH 7.4 (0.005 M Na_2HPO_4–0.001 M KH_2PO_4 adjusted to pH 7.4 with NaOH), pH 8.0 (0.080 M Tris–0.100 M$CaCl_2$ adjusted to pH 8.0 with HCl) and pH 10.0 (0.012 M Na_2CO_3–0.008 M $NaHCO_3$ adjusted to pH 10.0 with NaOH), at 37 ± 0.1 °C (Ulbrich et al., 2004; Zacchigna et al., 2003). PEG–Met was first put into dialtic bag and sealed, then was immerged into a buffer solution of 40 ml. After a suitable interval time, 5.0 ml of the solution released was withdrawn and another 5.0 ml of fresh buffer solution was added for maintaining the system to a stable volume (Liu et al., 2004). Standard curves were made by pure metaxalone (99.9%, w/w) in various buffer solutions. The concentration of the metaxalone released was analyzed and calculated by comparing the slopes of the standard curves at 271.0 nm. Each experiment was repeated three times.

2.7. Enzymatic hydrolysis study

The hydrolytic stability of PEG–Met to α-chymotrypsin was assessed in 0.08 M Tris buffer, 0.1M $CaCl_2$ at pH 8.0 buffer solution (Zacchigna et al., 2002; Giammona et al., 1998). Two hundred microliters of a α-chymotrypsin 10^{-5} M solution plus 0.001 M HCl were added into 2 ml of PEG–Met solution containing 7.1 mg of metaxalone, and then was put into dialtic bag and sealed, incubated in a buffer solution of 40 ml at 37 ± 0.1 °C. After a suitable interval time, 5.0 ml of the solution released was withdrawn and 5.0 ml of fresh buffer solution was added for maintaining the system with a stable volume (Liu et al., 2004). Standard curve was made by pure metaxalone (99.9%, w/w) in pH 8.0 buffer solutions. The concentration of the metaxalone released was analyzed and calculated by comparing the slopes of the standard curves at 271.0 nm. Each experiment was repeated three times.



Scheme 1. Schematic synthetic route of PEG–Met.

Table 1
Effect of the reaction condition on the synthesis of prodrugs

Sample	Type of prodrug	Temperature (°C)	Time (h)	Yield (%)
a	PEG_{4000}–Met	50	9	46.0
b	PEG_{4000}–Met	50	12	60.3
c	PEG_{4000}–Met	50	15	81.0
d	PEG_{4000}–Met	50	18	80.9
e	PEG_{4000}–Met	60	15	87.3
f	PEG_{4000}–Met	70	15	76.6
g	PEG_{4000}–Met	90	15	63.0
h	PEG_{6000}–Met	60	15	88.5
i	PEG_{10000}–Met	60	15	83.6

3. Results and discussion

3.1. Synthesis of PEG–Met conjugate

Metaxalone can chemically react with PEG by utilizing a chloroacetyl chloride linkage. In order to perform this synthesis, a preliminary reaction of metaxalone with chloroacetyl chloride to obtain an intermediate of *N*-chloroacetyl-metaxalone is necessary. Then PEG–Met conjugate is synthesized by reaction of the intermediate with sodium PEG, which can be synthesized by treatment of PEG with metal sodium. The detailed chemical route is showed in Scheme 1.

The effect of reaction condition on the synthesis of PEG–Met is shown in Table 1. The yield of PEG_{4000}–Met conjugate increases with the increase in reaction temperature from 50 to 60 °C, and the yield of PEG_{4000}–Met can even reach to the maximum of 87.3% at 60 °C for 15 h. With further increase in reaction temperature, the conversion of PEG_{4000} decreases. Meanwhile, the color of the prodrug becomes fuscous, and the separation of polymer prodrug faces difficulty because of more byproducts. The yield of PEG_{4000}–Met also increases with the increase in reaction time from 9 to 15 h. The maximum yield can be achieved after 15 h.

Additionally, thin layer chromatography and DSC can be used for determining free metaxalone in polymer prodrug, and the results as shown in Fig. 1. As can be seen from Fig. 1, pure metaxalone's melting point (a) is at 124.6 °C on its DSC-thermogram, however, no peak is visible near the metaxalone's melting point for three polymer prodrugs. It can also be clearly observed that the melting point for PEG_{4000}–Met (b) is 53.5 °C, for PEG_{6000}–Met (c) is 55.6 °C and for PEG_{10000}–Met (d) is 60.9 °C, respectively.

3.2. The mechanism of the hydrolysis of prodrug PEG–Met

As is well known, the general stability order of some chemical bonds to hydrolysis is ether > amide > ester. Oxazolidinones are relatively stable chemical entities under ordinary conditions. They are stable to acidic, mildly basic, and thus amide bond outside the oxazolidinone ring was first opened when PEG–Met prodrug underwent hydrolysis. Several possible routes for the hydrolysis of PEG–Met prodrug theoretically are shown in Scheme 2. PEG–Met hydrolyzed into metaxalone by processes (a). Metaxalone sequentially hydrolyzed into compounds (3 and 4) by processes (b and c). In addition, PEG-Met also possibly hydrolyzed into compound (5) by processes (d), then translated into compound (4) by the hydrolysis of amide bond.

Hydrolysis experiment of PEG–Met prodrug was processed to identify above steps. PEG_{6000}–Met was first put into dialtic bag and sealed, then was immersed into a buffer solution at pH 7.4 in presence of α-chymotrypsin for 8 h, hydrolysis products were analyzed by GC–MS to show the presence of only metaxalone (2) in solution. Other hydrolysis products such as compounds (3 and 4) did not exist in solution, and therefore PEG-Met was just hydrolyzed by processes (a) on the condition of simulative body fluid.

3.3. Controlled release in vitro

In order to obtain some preliminary information about the potential use of the PEG–Met as a drug delivery system for a

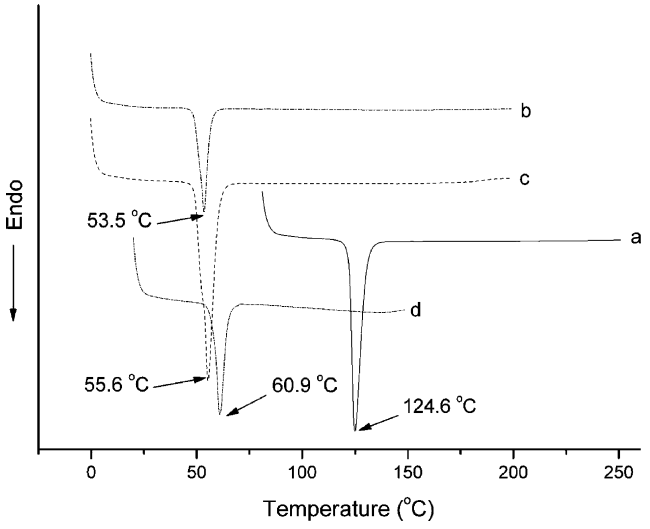


Fig. 1. DSC-thermograms of pure metaxalone (a), PEG_{4000}–Met (b), PEG_{6000}–Met (c) and PEG_{10000}–Met (d).

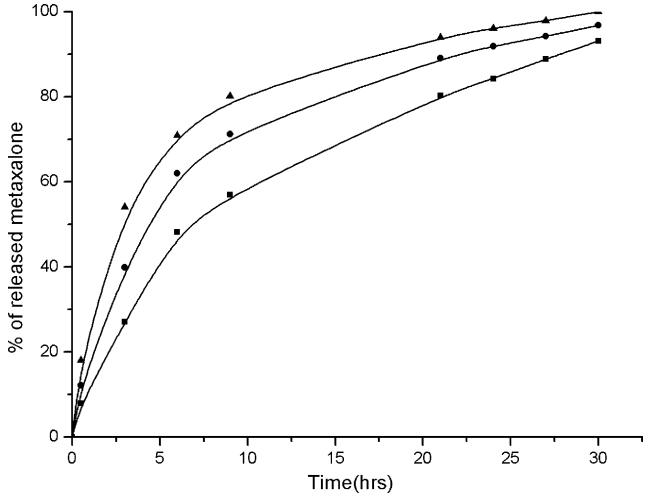
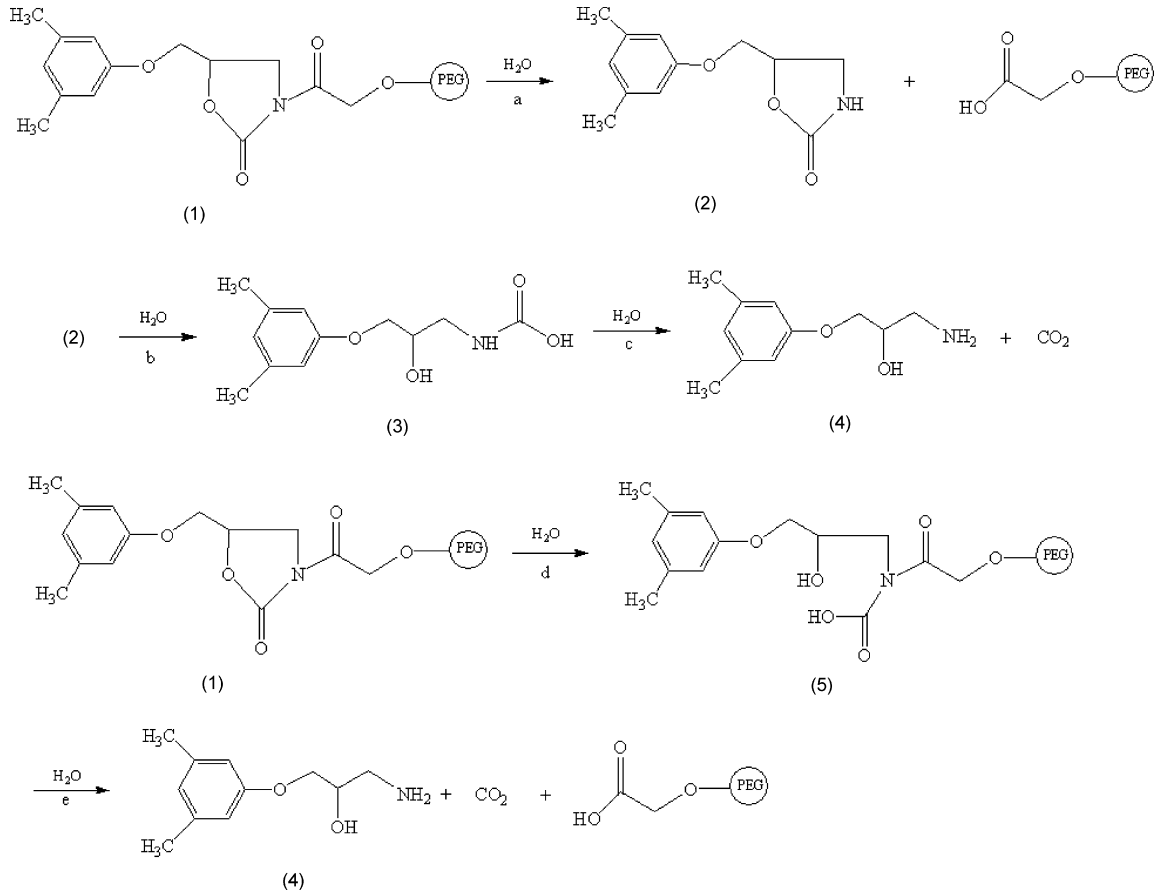


Fig. 2. Release of metaxalone in buffer solution, pH 1.1 (■), pH 7.4 (●), pH 10.0 (▲), at 37 °C from PEG_{4000}–Met.



Scheme 2. Possible routes for the hydrolysis of PEG–Met.

prolonged release, hydrolysis process for three prodrugs in vitro is studied in buffer solution at pH 1.1, 7.4, 10.0, and at pH 8.0 in the presence and in the absence of α-chymotrypsin.

Fig. 2 presents the hydrolysis rates of PEG_{4000}–Met at three pH levels. As can be seen PEG_{4000}–Met is subjected to hydrolyzation to 57.0% at pH 1.1 and 71.2% at pH 7.4 after 9 h, hydrolyzation percentage can reach to 80.2% at pH 10.0. Fig. 3 depicts the hydrolytic behavior for PEG_{6000}–Met. Clearly, at pH 1.1 the hydrolyzation percentage can reach to 54.3%, and at pH 7.4 and 10.0, the hydrolyzation percentage can reach to 61.6 and 69.5%, respectively. Fig. 4 depicts the same hydrolytic behavior for PEG_{10000}–Met. The hydrolyzation percentage is

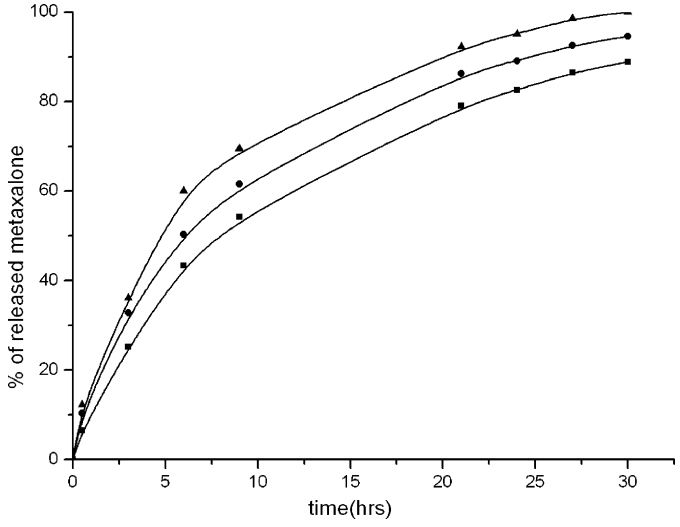


Fig. 3. Release of metaxalone in buffer solution, pH 1.1 (■), pH 7.4 (●), pH 10.0 (▲), at 37 °C from PEG_{6000}–Met.

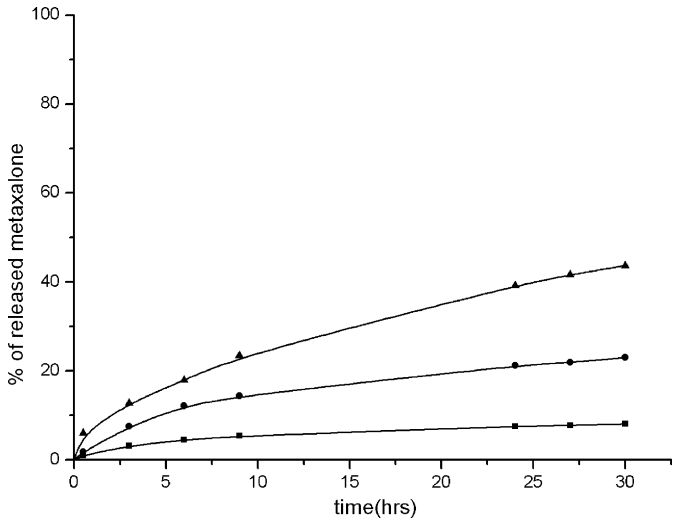


Fig. 4. Release of metaxalone in buffer solution, pH 1.1 (■), pH 7.4 (●), pH 10.0 (▲), at 37 °C from PEG_{10000}–Met.

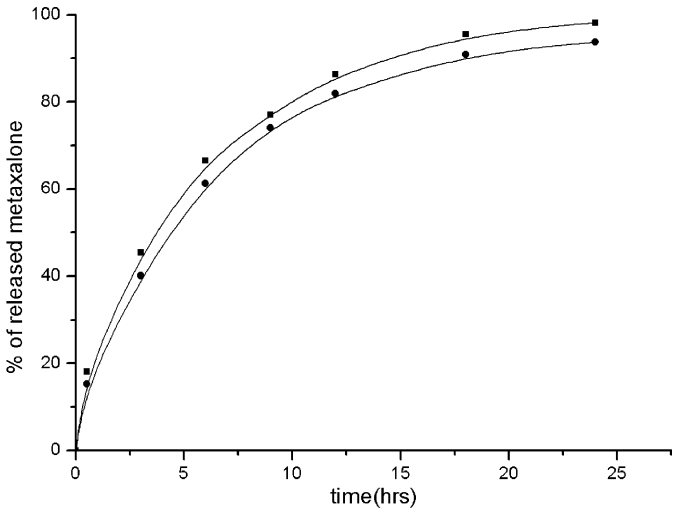


Fig. 5. Release of metaxalone from PEG_{4000}–Met in 0.08 M Tris butter at pH 8.0 in presence (■) and in absence (●) of α-chymotrypsin.

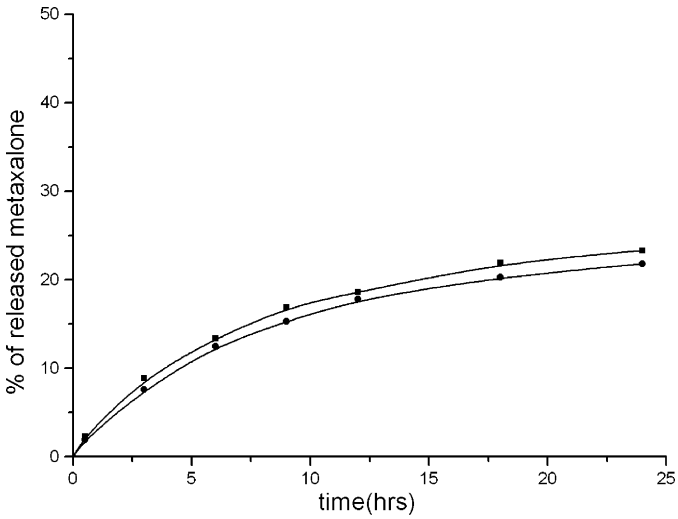


Fig. 7. Release of metaxalone from PEG_{10000}–Met in 0.08 M Tris butter at pH 8.0 in presence (■) or in absence (●) of α-chymotrypsin.

5.4% at pH 1.1, and 14.4 and 23.4% at pH 7.4 and 10.0, respectively.

The hydrolysis rates of metaxalone from three prodrugs are all relatively faster within 6 h. The hydrolysis rates of metaxalone from PEG_{4000}–Met, PEG_{6000}–Met and PEG_{10000}–Met are calculated to be 8.0, 7.2 and 0.75% per hour at pH 1.1, 7.4, respectively. The hydrolysis rates are calculated to be 10.3, 8.4 and 2.0 at pH 10.0. The hydrolysis rate of metaxalone from PEG_{10000}–Met is the slowest among three prodrugs in the same condition.

Besides, α-chymotrypsin, one of the digestive enzymes secreted by the pancreatic, belongs to a family of enzymes that can cleave proteins into small peptides. It is also widely known for its ability to catalyze the hydrolysis of ester bonds and amide bonds (Harada et al., 2000; Fersht, 1972). In order to understand the effect of α-chymotrypsin, the releases of metaxalone from PEG–Met prodrugs in the presence and in the absence of α-chymotrypsin at pH 8.0 are studied for determining the release rates.

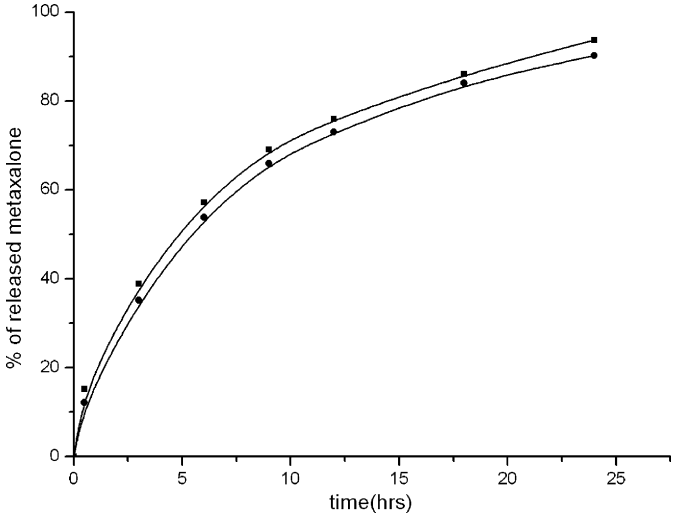


Fig. 6. Release of metaxalone from PEG_{6000}–Met in 0.08 M Tris butter at pH 8.0 in presence (■) or in absence (●) of α-chymotrypsin.

As can be seen from Fig. 5, 98.2% of metaxalone is released from PEG_{4000}–Met after 24 h in the presence of α-chymotrypsin, and 93.8% of metaxalone is released in the absence of α-chymotrypsin. The hydrolysis rates of metaxalone from PEG_{4000}–Met are calculated to be 11.1 and 10.5% per hour at pH 8.0 with and without α-chymotrypsin within 6 h.

As can be seen from Fig. 6, 93.7% of metaxalone is released from PEG_{6000}–Met in the presence of α-chymotrypsin, and 90.2% is released in the absence of α-chymotrypsin after 24 h. The hydrolysis rates of metaxalone from PEG_{6000}–Met are calculated to be 9.5 and 9.0% per hour at pH 8.0 with and without α-chymotrypsin within 6 h.

In Fig. 7, it can be seen that 24.8% of metaxalone is released from PEG_{10000}–Met in the presence of α-chymotrypsin, and 23.8% is released in the absence of α-chymotrypsin after 24 h. The hydrolysis rates of metaxalone from PEG_{10000}–Met are calculated to be 2.2 and 2.1% per hour at pH 8.0 with or without α-chymotrypsin within 6 h.

Based on the experimental results, more metaxalone can be released in the presence of α-chymotrypsin than in the absence according to the same testing condition. However, there exists a little difference between the release rates of metaxalone from the three prodrugs with or without α-chymotrypsin. Interestingly, it is clear that PEG_{10000}–Met is the most stable prodrug in various buffer solutions and in the presence or in the absence of α-chymotrypsin. These results suggest that the amide bond between metaxalone and PEG_{4000} may be easily susceptible to its environmental condition.

4. Conclusions

Three novel polymer prodrugs can be prepared via linking metaxalone to PEG possessing different molecular weights using chloroacetyl chloride as a spacer. The yield for PEG_{4000}–Met can reach to the maximum of 87.3% at 60 °C

for 15 h. PEG based prodrugs exhibited effective increases in solubility of metaxalone, while the metaxalone molecules bonding via an amide bond can guarantee the drug release. Three prodrugs were found to be relatively stable in different pH solutions by inspection of their releasing behaviors in vitro, and can slowly release the active drug molecules. The hydrolysis rate for PEG_{10000}–Met is the slowest among three polymer prodrugs at the same conditions. Thus, PEG_{4000}–Met was shown to be more susceptible to pH values. In the same buffer solution and time period, more metaxalone can be released in the presence of α-chymotrypsin than in its absence. Three prodrugs can markedly improve the pharmacokinetics of metaxalone, particularly, increasing its half-life.

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