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SN-38:β-cyclodextrin inclusion complex for *in situ* solidifying injectable polymer implants

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Abstract— One of the most useful techniques to treat cancer is chemotherapy. However, anticancer drugs, such as SN-38, have limited solubility with strong side effects. This work aims to use SN-38:β-cyclodextrin (β-CD) inclusion complex for an injectable polymeric in situ forming implant containing poly(ethylene glycol) (PEG), poly(ε-caprolactone), and poly(D,L-lactide). It was found that implant formation and SN-38 encapsulation efficiency directly depended on weight ratio of SN-38 and β-CD. At the ratio of SN-38:β-CD of 1:7, the implant could not be formed perfectly and had lower encapsulation efficiency. Reduction of the amount of β-CD to the ratio of 1:3 showed the higher encapsulation efficiency at 89.7 %. SN-38 release rate was also found to depend on β-CD content and the implant weight. In addition, their active form was protected when encapsulated inside implants.

Keywords— Polymeric drug delivery, Cancer, Cyclodextrin, Injectable implant, SN-38

I. Introduction

SN-38, 7-ethyl-10-hydroxycamptothecin, is a broad spectrum anticancer drug which is a derivative of camptothecin. SN-38 is 100 to 1,000 fold more active than camptothecin [1] which integrates into DNA structure, then induces apoptosis in both of a multidrug-resistance and a wild type human glioblastoma cell line [2]. Moreover, an active form of SN-38 (lactone form) can complex to topoisomerase I as an enzyme inhibitor. Although SN-38 has shown good activity for *in vitro* and *in vivo* experiments, low solubility and severe side effects limits its applications. Therefore, tremendous efforts have been made to improve the solubility, stability, or bioavailability [3]-[5]. SN-38 has as low as 36 μg/mL solubility in phosphate-buffer and there were also reports on the conversion of active form (lactone form) to inactive form (carboxylate form or open lactone ring) in aqueous. Moreover, SN-38 has a short half-life in blood circulation and is unstable in basic pH solution [4], [6]. Thus, the attempts have been shifted towards more controlled and stable systems such as local drug administration by loading drugs into polymers [7].

Local delivery has been used as an alternative approach for the transport of therapeutic agents and drug delivery systems across blood-brain barrier (BBB) through the brain. There are many reports concerning injectable implants using natural or synthetic polymers [8]-[9]. Herein, we reports the development of injectable polymeric in situ forming implants for SN-38 delivery to human gliomas using a biocompatible solvent (glycofurol) and a biodegradable copolymers, ([poly(ε-caprolactone)-random-poly(D,L-lactide)]-block-poly(ethylene glycol)-block-[poly(ε-caprolactone)-random-poly(D,L-lactide)]) or PLECs [10]-[11]. This drug delivery system could solidify after injection allowing the formation of small or irregular shapes according to targeted sites.

Cyclodextrin (CD), cyclic oligosaccharides, is used in many research fields such as food, chemical reagents, agriculture and pharmaceutical [12]. Cyclodextrin can be classified into 3 main types, α-CD, β-CD, and γ-CD constituted by 6, 7 and 8 units of glucopyranoside, respectively (the solubility of α-CD, β-CD, and γ-CD are approximately 145, 18.5, and 232 mg/mL). CDs have extensively used to enhance the solubility of the poorly soluble drugs including anticancer drugs such as β-lapachone [13], camptothecin [14]-[15], doxorubicin [16], and SN-38 [17]. Camptothecin inclusion complex with β-CD increased the solubility up to 171 times and the half-life was found to be 10 times higher than free drugs [14].

In this study, we described the preparation of SN-38:β-CD inclusion complex then encapsulated into PLEC implants for SN-38 delivery. The complex was mixed with biodegradable polymer, PLECs, then implants were formed. Physical properties of the implants including SN-38 encapsulation efficiency and *in vitro* drug release were characterized. High-performance liquid chromatography (HPLC) and thin layer chromatography (TLC) were used to analyze the stability of SN-38. The effect of the ratios of SN-38 to β-CD in *in vitro* experiment was discussed.

II. Materials and Experimental Procedures

A. Materials

7-ethyl-10-hydroxycamptothecin, SN-38, was obtained from Abatra technologies Co. Ltd. (Xian, China). Glycofurol (GF) and tin (II) 2-ethylhexanoate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Cyclodextrin (CD; α-CD, β-CD and γ-CD) was obtained from Cyclodextrin Technologies Development Inc. (Florida, USA). D,L-Lactide (LA), ε-caprolactone (CL) and poly(ethylene glycol)

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(PEG, M_W = 1000), were obtained from Acros Organics (New Jersey, USA.). Dimethyl Sulfoxide (DMSO) was obtained from RCI Lab-scan Ltd. (Milwaukee, USA).

B. Polymerizations

Copolymers with CL, LA and PEG (PLECs) were synthesized as previously reported [18]. The final ratio of CL, LA and PEG in copolymers was calculated and weighed. Then the mixture was added in the dried round-bottomed flask under dried argon and reduced pressure for 6 hours. Toluene was added as a solvent. The flask was immersed in an oil bath and maintained at 130 ^{o}C for 48 hours. Tin (II) 2-ethylhexanoate, a catalyst, was added to catalyze the polymerization. Polymer purification, copolymers were precipitated by cold methanol then they were analyzed by nuclear magnetic resonance spectroscopy (NMR).

C. Preparation of SN-38:β-CD Inclusion Complex

The inclusion complexes of SN-38 and b-CD (SN-38:β-CD) at the weight ratio of 1:1, 1:1.5, 1:3, and 1:7 were gently mixed with ethanol:water (1:2). The mixture was acidified by 0.02 N HCl against water volume and stirred under vacuum for 24 hours to evaporate ethanol. Finally, the solutions were added fresh water, frozen and freeze-dried [19]-[20].

D. Preparation of Polymer Implants

The blank PLEC solution and SN-38 loaded PLEC solution were then injected into a vial containing 10 mL of phosphate buffer saline (PBS, pH 7.4) at 37 ^{o}C. The amount of SN-38:β-CD inclusion complex and polymer are listed in Table I. Glycofurol volume was fixed at 100 μL. These PLEC implants were immediately formed by injecting into phosphate buffer saline. Unloaded SN-38 and SN-38 releases were measured spectrophotometrically at 384 nm. Equation (1) was used to determine the SN-38 encapsulation efficiency. It should be noted that the amount of SN-38 in polymer implant was determined by substracting the unloaded SN-38 from the initial amount of SN-38.

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Amount of SN-38 in polymer implant} \times 100}{\text{Initial amount of SN-38 added to formulation}} \quad (1)$$

TABLE I
COMPOSITION OF SN-38:β-CD IMPLANTS

SN-38 and β-CD ratio	SN-38:β-CD inclusion complex (mg)	PLEC (mg)
1:1	9.00	21.00
1:1.5	11.25	18.75
1:3	9.00	21.00
1:7	9.00	21.00

E. Release study

Implants were placed and kept at 37 ^{o}C with continuously orbital shaking at 90 rpm. At the predetermined time, all of PBS buffer was removed then fresh buffer was added in the vial and kept at 37 ^{o}C. The removed buffer was determined for the amount of SN-38 by spectrophotometer as previously described. It should be noted that 4 mL of DMSO was added to solubilize the precipitated drug or polymers. The cumulative amount of drugs was calculated by equation (2). The release profiles were obtained by plotting the amount of released against time.

$$\text{Cumulative SN-38 released (\%)} = \frac{\text{Amount of SN-38 release} \times 100}{\text{Initial amount of loaded SN-38}} \quad (2)$$

F. HPLC and TLC Analysis of Active and Inactive Form of SN-38

The removed PBS buffer was also analyzed by HPLC system (Waters, Manchester, UK) equipped with UV detector at 265 nm and a reverse-phase C18 column (150 mm × 4.6 mm, 5μm particle size). A mobile phase consisted of 25 mM of NaH_2PO_4, pH 3.1 and acetonitrile, with 70/30 ratio. For TLC analysis, the removed PBS buffer was spotted on a TLC plate (TLC silica gel 60 F_{254}, Merck, Darmstadt, Germany), then TLC plates were partially submerged in a mobile phase, $CHCl_3$: MeOH: Acetone (9:1:1). Plates were dried before characterization under UV light.

G. Statistical Analysis

All experiments were performed in triplicate. Data was reported as mean ± standard deviation. An unpaired Student's *t*-test was used for data analysis. A statistically significant difference was defined at the 95% confidence level.

III. RESULTS AND DISCUSSION

A. Polymeric Implants Formulation

PLEC copolymers were synthesized with 21.5% mole of D,L-lactide compared to ε-caprolactone. The molecular weight of this PLEC was at 56.1 kDa with the molecular weight of PEG at 1 kDa. PLEC mole ratio and molecular weight was calculated by NMR. After dissolving SN-38:β-CD inclusion complex and PLEC copolymer in glycofurol, the mixture was gently injected into PBS buffer where glycofurol and cyclodextrin can spontaneously dissolve in buffer after injection leaving drugs within solid polymer implants. For the SN-38 and β-CD ratio of 1:1.5, the implants contained the lowest polymer concentration (18.75 mg) which could not form stable implants and disappeared within 30 minutes. Comparing to blank polymer implants, introduction of SN-38 into this formulation raised hydrophobicity of implants which led to stable implants.

Figure 1 shows the solidification of PLEC implants with and without SN-38:β-CD inclusion complex. After 30 days of incubation, implants degraded in the layer by layer

fashion resulting in the release of SN-38:β-CD inclusion complex. The blank implants degradation rate was faster than SN-38:β-CD implants. This was also a result of increasing in implant hydrophobicity by SN-38. In the encapsulation experiment, SN-38:β-CD inclusion complexes with the ratio of 1:1, 1:3, and 1:7 were encapsulated in PLEC implants (Table II). Low encapsulation efficiencies were observed when 1:7 SN-38:β-CD inclusion complex was used where the other two formulations had as high as 89% of SN-38 encapsulation efficiency.

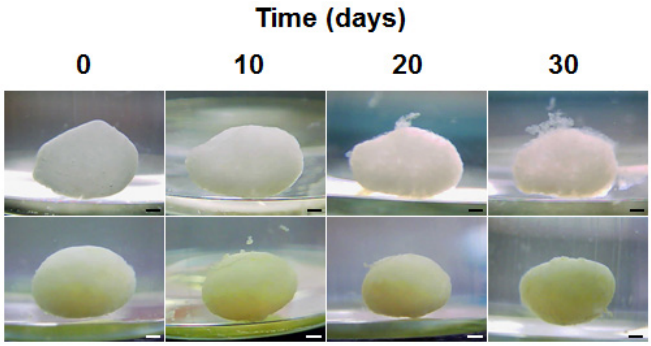

Fig. 1. Photo sequence of implants at different times. The implants consisting of PLEC (56.1 kDa) and 1:3 of SN-38:β-CD, were formed in PBS pH 7.4 at 37 °C. Upper row shows the blank implants while lower row shows SN-38:β-CD inclusion complex loaded implants. The scale bar is 1 mm.

TABLE II
FORMULATION OF POLYMER IMPLANTS WITH DIFFERENT RATIO OF β-CD

SN-38 and β-CD ratio	Implant formation characteristics	SN-38 Loading (mg)	% EE	Implant weight (mg)
1:1	+,VV	1.45 ± 0.14	89.7 ± 0.8	40.0 ± 5.3
1:1.5	-,V	-	-	-
1:3	++,VV	0.77 ± 0.01	89.7 ± 3.7	47.3 ± 2.2
1:7	+,V	0.21 ± 0.06	57.0 ± 9.0	44.9 ± 4.1

(-) = The implants are not formed. (+) = The implants are formed as an irregular shape. (++) = The implants are formed as a spherical shape. (V) and (VV) represents low and high viscosity of the mixture solution, respectively. % EE is % encapsulation efficiency.

B. In Vitro SN-38 Release Profile

The release profiles were carried out for SN-38:β-CD inclusion complexes at the ratio of 1:3 and 1:7 where the implants were formed as spherical shape. The implants contained 1:7 ratio showed the highest release rate by diffusion mechanism. SN-38 released from implants with 1:3 and 1:7 ratio at 16.2 and 51.5%, respectively within 30 days as shown in Figure 2. Close observation revealed that β-CD and glycofurol released out while the implants was solidifying. This produced a lot of pores on implant surfaces, especially for 1:7 ratio which led to low encapsulation efficiency (57.0 ± 9.0 %) of SN-38. Hence, 1:3 ratio of SN-38:β-CD inclusion complex with comparatively higher encapsulation was used in the release study.

Figure 3 shows the effect of the implant weight on the release profile. The PLEC implants with different weight were prepared at 19.3 ± 0.5, 30.5 ± 2.6, and 47.3 ± 2.2 mg. The release profiles of these implants showed two phase release profile, i.e., the initial burst release of SN-38 followed by slow and constant release rate. Smaller implants clearly showed faster release rate and SN-38 release from 19.3 mg implants was found to be 2-fold higher than that of 47.3 mg implants at 30 days after incubation.

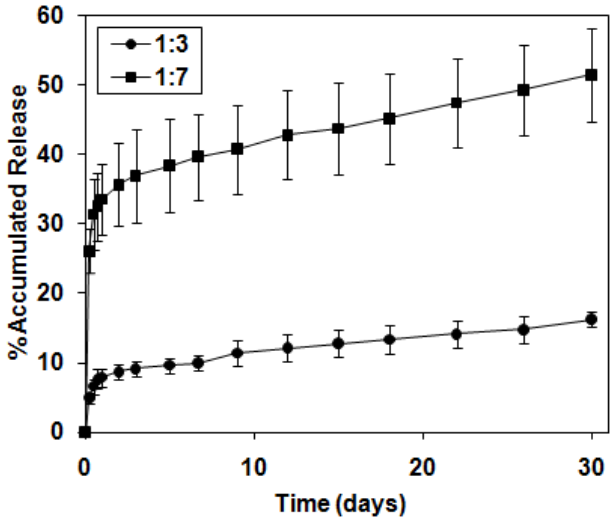

Fig. 2. Release profiles of SN-38 from the implants in PBS, pH 7.4, with different ratio of SN-38:β-CD inclusion complex; 1:3, 47.3 ± 2.2 mg (●) and 1:7, 44.9 ± 4.1 mg (■).

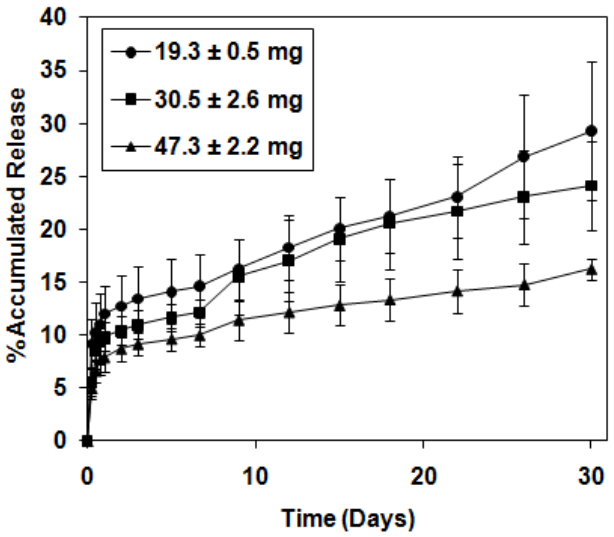

Fig. 3. Release profile of SN-38 from the implants in PBS, pH 7.4. SN-38:β-CD inclusion complex 1:3 ratio was used with various implant weights; 19.3 ± 0.5 mg (●), 30.5 ± 2.6 mg (■), and 47.3 ± 2.2 mg (▲).

C. Stability of SN-38

In aqueous, SN-38 can be in both active and inactive form. The inactive form occurred when the lactone ring of SN-38 was hydrolyzed and become the carboxylate form. Because of SN-38 half-life in PBS is 16 minutes, the qualitative characterization of active and inactive form of the released SN-38 was carried out by HPLC and TLC [4]. As shown in Figure 4, TLC showed that the majority of SN-38 was in the active form (R_f ~ 0.44) at day 10, 20 and 30. It should be noted that the R_f of inactive form was 0. HPLC analysis revealed that 13.9 and 29.9% of SN-38, in 30.5 $\pm$ 2.6 and 47.3 $\pm$ 2.2 mg implants were converted to the carboxylate at day 30. These results confirmed that the implants could protect lactone ring from the hydrolysis and are suitable for the controlled release application of unstable drugs like SN-38.

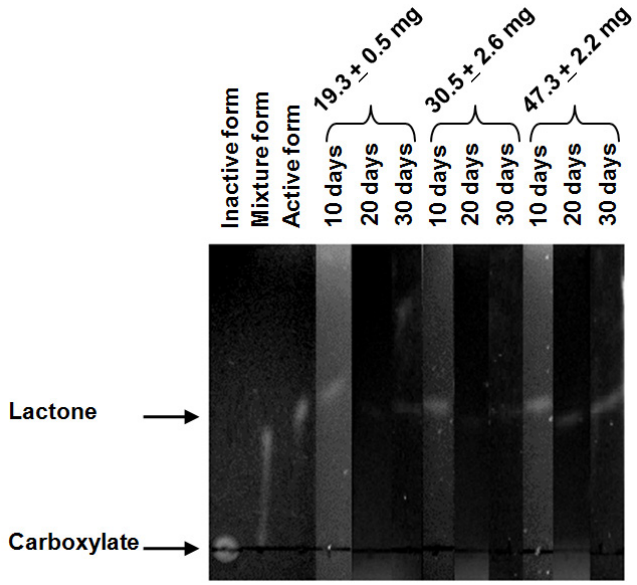

Fig. 4. Thin-layer chromatography (TLC) analysis of the lactone and carboxylate form of SN-38 at different times.

IV. Conclusion

The SN-38:β-CD inclusion complex was successfully encapsulated in implants using PLEC copolymers as materials. The implants showed high encapsulation efficiency of SN-38. The SN-38:β-CD inclusion complex enhanced drug properties, stability and solubility. At lower implant weight, the release rate was found to be faster. We believe that these implants are a choice for cancer treatment, particularly high-grade gliomas.

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