International Journal of Pharmaceutics 397 (2010) 116–121

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Complex of 9-nitro-camptothecin in hydroxypropyl-β-cyclodextrin: In vitro and in vivo evaluation

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ARTICLE INFO

Article history:
Received 21 April 2010
Received in revised form 1 July 2010
Accepted 8 July 2010
Available online 15 July 2010

Keywords:
9-Nitro-camptothecin
Hydroxypropyl-β-cyclodextrin
Freeze drying
Stability
Dissolution
Pharmacokinetic studies

ABSTRACT

The effect of a series of cyclodextrins (CDs), especially hydroxypropyl-β-cyclodextrin (HP-β-CD), on aqueous solubility and chemical stability of 9-nitro-camptothecin (9-NC), was investigated with an aim of preparing a stable and effective parenteral formulation. The 9-NC/HP-β-CD complex was obtained in solid form by freeze drying. Then, the pharmacokinetic profiles in rats of aqueous complex were compared to those of free 9-NC solution having an equivalent concentration. The aqueous solubility of 9-NC was increased to 0.52 mg/ml (lower than 5 μg/ml in distilled water, 25 °C) by the combination of pH and temperature adjustment. In addition, hydrolysis of 9-NC following pseudo-first-order kinetics was decelerated significantly in physiologic condition in the presence of HP-β-CD. Comparison of in vivo pharmacokinetic parameters of free 9-NC with the complex indicated that the complex had higher $AUC_{0-\infty}$ (439.39 ng h/ml vs. 632.79 ng h/ml for i.m. administration and 385.39 ng h/ml vs. 538.05 ng h/ml for i.v. administration, respectively) and ratio of lactone form. These results demonstrated that 9-NC/HP-β-CD complex is an attractive parenteral formulation for cancer therapy.

1. Introduction

9-Nitro-camptothecin (9-NC) is a potent topoisomerase-I inhibitor. Pharmacological studies indicated that the anti-tumor activity of 9-NC was superior to that of camptothecin (CPT) in human tumors xenografted in nude mice (Gao et al., 2008). However, the clinical application of 9-NC was largely hampered because of its poor solubility (lower than 5 μg/ml in distilled water, 25 °C) and stability (Verschraegen et al., 1998), which led to low therapeutic efficacy and a number of side effects such as neutropenia, thrombocytopenia, and diarrhea owing to the conversion of 9-NC from active lactone form to the inactive carboxylate form under physiologic conditions (illustrated in Fig. 1) (Haglof et al., 2006).

In recent years, to improve the stability and solubility of 9-NC, several formulation strategies have been investigated, including biodegradable polymer micelles (Gao et al., 2008; Han et al., 2009), nanoparticles (Dadashzadeh et al., 2008), liposome (Chen et al., 2006; Zhang et al., 2008) and self-microemulsifying formulation (Lu et al., 2008). However, up to now, none of the 9-NC formulations could be applied for clinical therapy. Therefore, the development of a novel parenteral formulation should be taken into consideration.

Cyclodextrins (CDs) are torus-shaped oligosaccharides consisting of 6, 7 or 8 (α-, β- and γ-CD, respectively) glucopyranose units through α-1, 4-linkages with hydrophobic central cavity and hydrophilic exterior surface. The entire or at least partial inclusion process of some drugs into CDs could lead to some improvements in a variety of physicochemical and pharmaceutical properties such as aqueous solubility, chemical stability and bioavailability of molecules (Loftsson and Brewster, 1996). Although β-CD is the most useful natural CDs for pharmaceutical applications since its central cavity has good affinity for the hydrophobic structures of many compounds, it is not always ideal for drug formulations due to its relatively low aqueous solubility (1.8% at 25 °C), renal toxicity and membrane destabilizing properties after parenteral administration. Recently, a number of chemically modified CDs have been prepared to improve the inclusion capacity and physicochemical properties of natural CDs. In particular, as the first cyclodextrin derivative proved by FDA, hydroxypropyl-β-cyclodextrin (HP-β-CD) has been extensively investigated due to its superior water-solubility and safety profile by the parenteral route as well as higher complexation potential relative to the parent β-CD (Irie and Uekama, 1997; Szente and Szejtli, 1999). It is worth noting that HP-β-CD can be found in marketed parenteral formulations (Loftsson and Duchene, 2007). Furthermore, commercial aqueous itraconazole formulations for i.v. administration contain HP-β-CD as high as 40% (w/v).

Several studies have reported complexation of CPT by cyclodextrins (Kang et al., 2002; Sætern et al., 2004). The solubility and stability of CPT were increased in the presence of CDs. However, to the best of our knowledge, there are no studies reported on complexation of 9-NC by CDs up to now. The main objective of this study

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doi:10.1016/j.ijpharm.2010.07.012

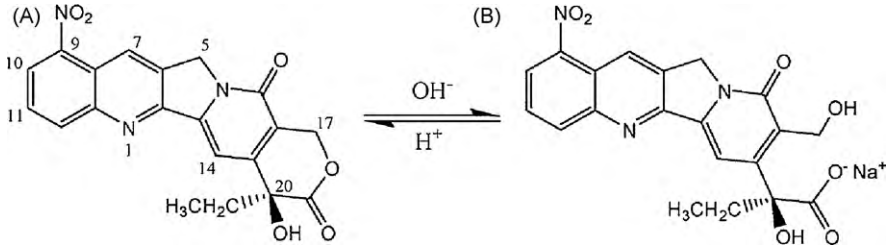

Fig. 1. The pH-dependence equilibrium between the lactone (A) and carboxylate (B) forms of 9-NC.

was to evaluate the effect of a series of cyclodextrins (CDs), especially hydroxypropyl-β-cyclodextrin (HP-β-CD) on the solubility, stability and pharmacokinetics in rats plasma of 9-NC.

2. Materials and methods

2.1. Materials

9-NC was provided by Ai Sike Biotechnolgy Co. Ltd. (Shanghai, China). The purity of 9-NC was 99.6%, which was verified by HPLC. CDs were supplied as described below: α-cyclodextrin(α-CD), β-cyclodextrin(β-CD), γ-cyclodextrin, γ-CD, and HP-β-CD (degree of substitution = 4.1–5.1) were provided by International Specialty Products Corporation as gift. Methyl-β-cyclodextrin (Me-β-CD) and glucosyl-β-cyclodextrin, Glu-β-CD, were purchased from Xinda Fine Chemical Co. Ltd. (Shandong, China). All other chemicals, including buffer components, were of analytical reagent grade. All aqueous preparations were made using double distilled water and organic solvents used in the analysis were high-performance liquid chromatography (HPLC) grade.

2.2. HPLC analysis

The analysis of lactone and carboxylate forms of 9-NC were performed using Shimadzu HPLC system with UV detection method, equipped with a C-18 analytical column (15 cm × 4.6 mm, 5 μl). The mobile phase consisted of a mixture of aqueous triethylamine–acetate buffer (prepared using 0.1%, v/v, triethylamine, adjusted with glacial acetic acid to pH 5.5) and acetonitrile (70:30, v/v) at a flow rate of 1.0 ml/min. The effluents were monitored at 368 nm and quantified by comparing the peak areas with the standard curve.

2.3. Determination of the apparent stability constants by solubility studies

The solubility measurement was carried out according to the method of Higguchi and Lach (1954). 2 mg 9-NC was suspended in 3 ml pH 5.0 phosphate buffer solution (PBS) containing different amount of CDs. The mixture was sonicated for 30 min and then incubated in a shaking water bath at 25 ± 0.5 °C for 48 h. The supernatant was filtrated through 0.45 μm filter, the first drops were discarded, and the rest collected and upon dilution, analyzed by HPLC.

2.4. Temperature effect

Excess amount of 9-NC was added to pH 5.0 PBS containing different amounts of HP-β-CD. After sonicating for 30 min, the mixture was shaken for 48 h until equilibrium was reached at different temperatures (25, 37 and 60 °C). Then, the solutions were centrifuged and the supernatant was then analyzed as described earlier.

2.5. pH effect

The pH effects on the solubility were carried out in various pH PBS (3.0, 4.0, 5.0, 6.0) at 25 ± 0.5 °C. Excess amount of 9-NC was added into the solution, sonicated for 30 min and then shaken for 48 h. Then, the solutions were centrifuged and the supernatant was then analyzed as described earlier.

2.6. Hydrolysis kinetics of 9-NC

The hydrolysis kinetic experiments were performed at 25 ± 0.5 °C in pH 7.4 PBS containing different amounts of HP-β-CD. 10 μl stock solution of 9-NC in dimethyl sulfoxide (DMSO) (1 mg/ml) were added into 2 ml PBS solution to produce an initial drug concentration of 5 μg/ml. The concentration of DMSO in the final solution was less than 1%. At predetermined time, 20 μl of the reaction solution was withdrawn and analyzed directly and immediately for both lactone and carboxylate forms of 9-NC by HPLC as described above.

2.7. Preparation of 9-NC complex

The inclusion complex of 9-NC with HP-β-CD was prepared by the colyophilization technique. 9-NC and cyclodextrin were dissolved in PBS (pH 5.0) in an appropriate molar ratio. The system was left to equilibrate under constant stirring for 2 h at 60 °C and protected from light. At the end of equilibrium time, the dispersion was filtrated, and the filtrate containing soluble complex was lyophilized to obtain dry yellow powder.

2.8. Dissolution studies

Dissolution profiles of free 9-NC and complex lyophilized product were evaluated according to Pharmacopoeia of the People's Republic of China 2010. Briefly, 1 mg free 9-NC or equivalent amount of complex was added to 0.9L hydrochloric acid (0.1 M) at 37 ± 0.5 °C by the paddle method at a rotation speed of 75 rpm. At predetermined times, aliquots (0.5 ml) were withdrawn, filtrated through 0.45 μm filter and analyzed by HPLC for 9-NC content.

2.9. Pharmacokinetics in rat plasma

Male Sprague–Dawley rats (180 ± 10 g) were fasted for 12 h before drug administration. 9-NC solution was composed of DMSO:polyethylene glycol (PEG) 400:ethanol:5% glucose (3:3:2:2 by volume) (Chen et al., 2007). The solution was prepared by first dissolving 9-NC in DMSO followed by the addition of the other solvents and immediately administered after preparation. Complex lyophilized product was re-dissolved in water. Four groups of rats were given 1.5 mg/kg 9-NC or complex solution via the tail vein or the thigh muscles, respectively. The blood samples (about 0.3 ml) were collected from tail vein at predetermined time intervals (5, 15, 30, 45, 60, 90, 120, 180 min) and centrifuged at 12,000 rpm for 2 min so as to separate the plasma. 200 μl ice-cold methanol

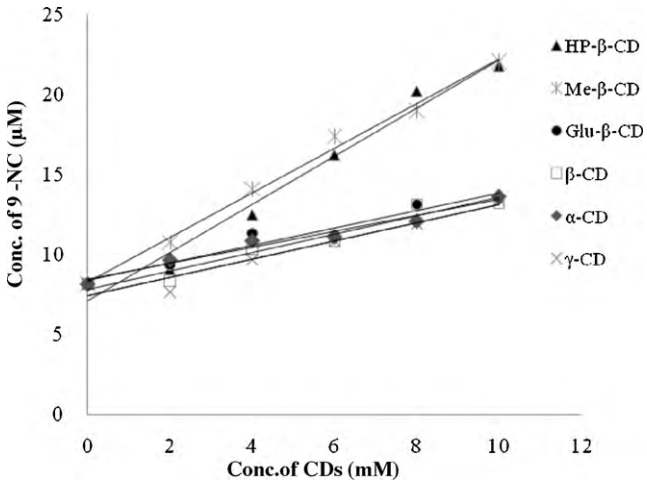

Fig. 2. Solubility of 9-NC in present of different concentration of CDs.

acetonitrile (1:1, v/v) was added to 100 μl plasma sample. The mixture was vortexed for 20 s and centrifuged at 12,000 rpm for 2 min. The supernatant was separated and stored at −20 °C until analysis. The rats were administrated with 0.5 ml saline orally every 0.5 h during the pharmacokinetic experiment to maintain the normal blood volume. All animal experiments were carried out in accordance with guidelines evaluated and approved by the ethics committee of Fudan University (Shanghai, China).

2.10. Analysis of 9-NC in plasma

The supernatant of the sample was injected into the HPLC system for the analysis of intact lactone 9-NC. For the determination of total 9-NC in plasma, 10 μl glacial acetic acid was added to 90 μl supernatant and the mixture was vortexed for 1 min and then analyzed by HPLC as described above (Chen et al., 2007).

2.11. Analysis

The pharmacokinetic parameters such as AUC (area under the drug concentration–time curve), t_{max} (time to reach the blood level peak after administration of the drug), $t_{1/2}$ (biological half life) were calculated using DAS 2.0 software. Results were expressed as mean ± SD for six rats. Statistical analysis was performed by Student's *t*-test for two groups, and one-way ANOVA for multiple groups. All results were expressed as the mean ± standard deviation (SD). A probability (*P*) of less than 0.05 is considered statistically significant.

3. Results and discussion

3.1. Comparison of various CDs

The solubilization capacity of various CDs to 9-NC is presented in Fig. 2 and Table 1. The solubility of 9-NC increased linearly with increasing concentration of various CDs, indicating the formation of a 1:1 complex. In this case, the apparent stability constant of the complex, K_c, can be calculated by the phase solubility method using the relationship: $K_c = \text{slope}/S_0(1 - \text{slope})$; S_0 were determined by the molar concentration of 9-NC in absence of CDs.

Table 1
Apparent stability constant of 9-NC/CD inclusion complex.

Cyclodextrin	S_0 (μM)	Slope ($\times 10^{-3}$)	*r*	K_c (M^{-1})
α-CD	8.16	0.499	0.983	61.2
β-CD	8.16	0.575	0.972	70.5
γ-CD	8.16	0.573	0.970	70.3
HP-β-CD	8.16	1.505	0.988	184.8
Me-β-CD	8.16	1.395	0.996	171.3
Glu-β-CD	8.16	0.548	0.975	67.2

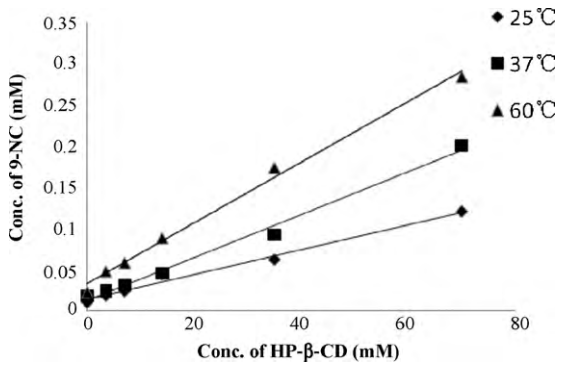

Fig. 3. Solubility of 9-NC with HP-β-CD at different temperature (25, 37 and 60 °C).

Among the naturally occurring CDs, β-CD showed the greatest solubilization capacity to 9-NC, probably due to its proper cavity size, allowing the guest molecular to move in easily but not move out. Among β-CD and its derivatives in this study, HP-β-CD solubilized 9-NC to the greatest extent ($K_c = 184.8\ M^{-1}$), followed by Me-β-CD ($K_c = 171.3\ M^{-1}$), approximately 2.5-fold of β-CD and Glu-β-CD. The different behavior of the four CDs may be considered in parallel with the solubility of the cyclodextrins employed, i.e., solubility of β-CD and Glu-β-CD were much lower than that of HP-β-CD and Me-β-CD. The significantly higher solubilizing effect of HP-β-CD could be attributed to the hydroxyl groups, which made the HP-β-CD molecule highly soluble in water. Furthermore, the recent studied indicated that the hydrogen bond formation between drug and cyclodextrin could further contribute to drug solubilization through non-inclusion complexation. Thus, it suggested that the effect of non-inclusion complexation in the solubilization ability of HP-β-CD was more evident than that of β-CD, resulting in the better solubilization ability of the former. It should be also taken into account that the low solubility of β-CD was attributed to the strong inter- and intra-molecular hydrogen bond formation between the secondary hydroxyl groups of CD molecules, while no such bonding was observed in the case of HP-β-CD due to partial substitution of the secondary hydroxyl groups of the CD molecule with hydroxypropyl groups (Maragos et al., 2009). Similar to HP-β-CD, the methyl groups of Me-β-CD disrupted intra-molecular hydrogen bonding, but also enlarged the whole cavity of the molecule by extending the secondary hydroxyl side and narrowing the primary hydroxyl side of the cone. Although Me-β-CD performed similar solubilizing effect as HP-β-CD, it formed stable complex with cholesterol, resulting in nephrotoxicity (Szejtli, 2004).

3.2. The temperature effect

The van't Hoff plot was obtained by plotting $\ln K_c$ against the reciprocal of absolute temperature (Figs. 3 and 4). The enthalpy ($\Delta_r H_m^\theta$) and the entropy ($\Delta_r S_m^\theta$) for the complexation were determined from slope of the plot, according to Eq. (1):

$$\ln K_c = \frac{\Delta_r H_m^\theta}{RT} + \frac{\Delta_r S_m^\theta}{R} \quad (1)$$

where *T* is the temperature and *R* is the gas constant. The $\Delta_r H_m^\theta$ and $\Delta_r S_m^\theta$ represent, respectively, the standard enthalpy and entropy of the 9-NC transference from the mobile phase to cyclodextrin cavity. For linear van't Hoff plot, the slope and the intercept are, respectively, $-\Delta_r H_m^\theta/R$ and $\Delta_r S_m^\theta/R$.

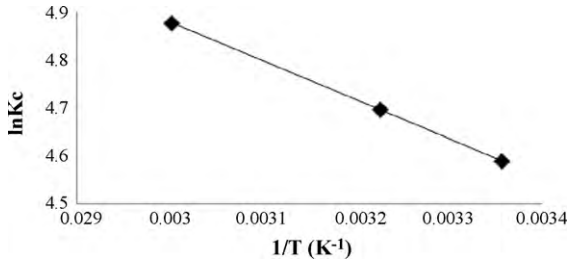

Fig. 4. van't Hoff plot of ln K_c vs. $1/T$ of complex.

Table 2
Thermodynamic parameters change in the formation of 9-NC/HP-β-CD complex.

T (K)	K_c (M^{-1})	$\Delta_r S_m^\theta$ (J/mol/K)	$\Delta_r H_m^\theta$ (J/mol)	$\Delta_r G_m^\theta$ (J/mol)
298	184.3	60.7	75885.46	−11366.1
310	142.5	60.7	83324.82	−12097.2
333	161.5	60.7	96818.45	−13492.2

Table 2 shows the thermodynamic parameters for inclusion complex formation of 9-NC and HP-β-CD. The enthalpy and the entropy of the reaction were both positive. Positive enthalpy value means that the system was receiving energy upon complexation accompanying dipoles and van der Waals interaction, which meant the reaction was tend to occur with increasing temperature. Positive entropy value indicated that the randomness of the overall system increased upon complexation. As the inclusion complex was formed, the water molecules surrounding cyclodextrin in orderly fashion were disrupted and squeezed out into a more randomized structure, leading to the increase in the entropy of the overall system. In addition, the Gibbs energy ($\Delta_r G_m^\theta$) was calculated from Eq. (2), and the negative value means the inclusion was a spontaneous process:

$$\Delta_r G_m^\theta = -RT \ln K_c \quad (2)$$

3.3. The pH effect

As displayed in Table 3, the solubility of 9-NC increased slightly with increasing pH. However, the apparent K_c values were found to decrease with increasing pH from 332.19 M^{-1} at pH 3.0 to 167.52 M^{-1} at pH 6.0. According to previous study (Chen et al., 2007), 9-NC tended to hydrolyze to the carboxylate form with increasing pH. At pH 6.0, part of 9-NC molecules existed as carboxylate form, which was highly water soluble, making the slight increase in S_0. On the other hand, the hydrophilic character of the carboxylate form of 9-NC reduced the driving force for inclusion into the apolar HP-β-CD cavity, resulting in a decrease of K_c.

3.4. Hydrolysis kinetics of 9-NC

The effect of HP-β-CD on the stability of 9-NC is depicted in Fig. 5. The inverse linear relationship obtained between the natural logarithm concentration of hydrolyzed 9-NC and time unambiguously indicated the hydrolysis of 9-NC, irrespective of the concentration of HP-β-CD, follows pseudo-first-order kinetics.

Table 3
Apparent stability constant of 9-NC/HP-β-CD inclusion complex in different pH phosphate buffer solution.

pH	S_0 (μM)	Slope ($\times 10^{-3}$)	r	K_c (M^{-1})
3.0	4.1	1.35	0.999	332.19
4.0	5.6	1.46	0.999	258.51
5.0	8.1	1.47	0.996	184.30
6.0	9.6	1.56	0.994	167.52

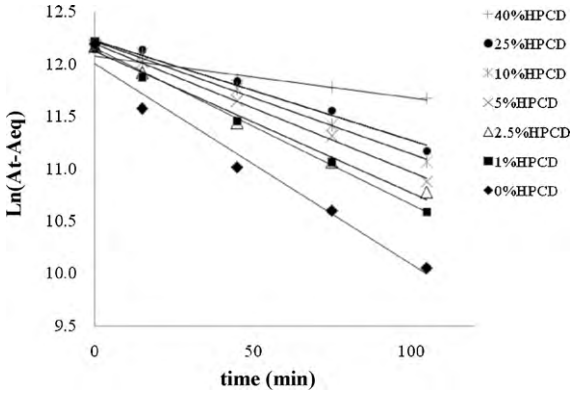

Fig. 5. Effect of HP-β-CD on the stability of 9-NC in pH 7.4 PBS.

The hydrolysis rate was calculated from the slope of Fig. 5. As shown in Table 4, the observed pseudo-first-order rate constant decreased with an increase in the concentration of HP-β-CD, indicating that the hydrolysis of 9-NC encapsulated in HP-β-CD is slower than that of 9-NC present outside the cavity. This exponential relationship between the hydrolysis rate of 9-NC and the concentration of HP-β-CD could be explained by saturation kinetics. The half life was approximately a 5-fold increase in the presence of 40% HP-β-CD. These results clearly showed that the hydrolysis of 9-NC in complex form is much slower than that of free 9-NC.

3.5. Preparation of 9-NC complex

The results of phase solubility indicated the formation of a 1:1 complex. However, the small complexation constant made the molar ratio between 9-NC and HP-β-CD in the solution relatively low, i.e. approximately 1/800 at therapeutic pH. This meant that at equilibrium <0.13% of the cyclodextrin molecules were occupied by the drug. After adjusting temperature and pH, the 9-NC concentration in water was 0.52 mg/ml (40% HP-β-CD, approximately 150-fold higher than that of free 9-NC), which could satisfy the clinical therapeutic dose.

3.6. Dissolution studies

The dissolution profiles of 9-NC and complex lyophilized product are illustrated in Fig. 6. At each time point, 9-NC amount dissolved from the lyophilized powder was significantly higher than that of free 9-NC.The amount dissolved from free 9-NC was less than 10% after 45 min, while the 9-NC amount dissolved from lyophilized powder was almost 100% in 5 min. This fast dissolution was owing to the fact that HP-β-CD have the capability of improving the wettability of powder and forming rapidly soluble complexes in solution. In addition, a decrease in crystallinity of the drug might be the factor in the enhanced dissolution by the complex. The fast dissolution of the complex is conducive to rapid preparation of clinical injection solution, without adding co-solvent or organic solvents.

Table 4
The hydrolysis rate in the presence of HP-β-CD in phosphate buffer solution (pH 7.4).

Conc. of HP-β-CD (g/ml)	Hydrolysis rate (min^{-1})	Half life (min)
0	0.019	36.5
1	0.015	46.2
2.5	0.013	53.3
5	0.012	57.8
10	0.01	69.4
25	0.009	77

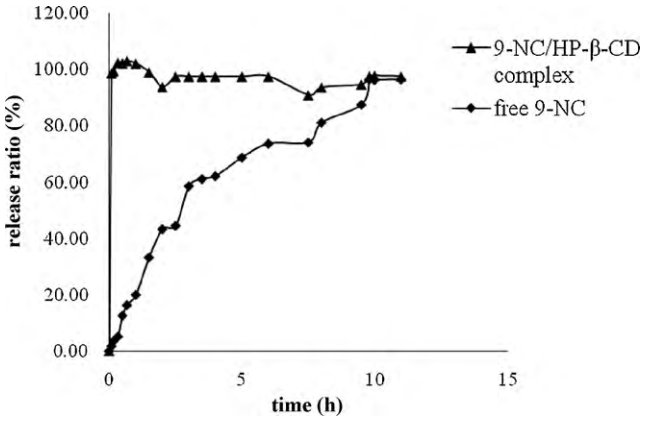

Fig. 6. Dissolve curve of free 9-NC and 9-NC/HP-β-CD complex in 0.1 M HCl at 37 ± 0.5 °C.

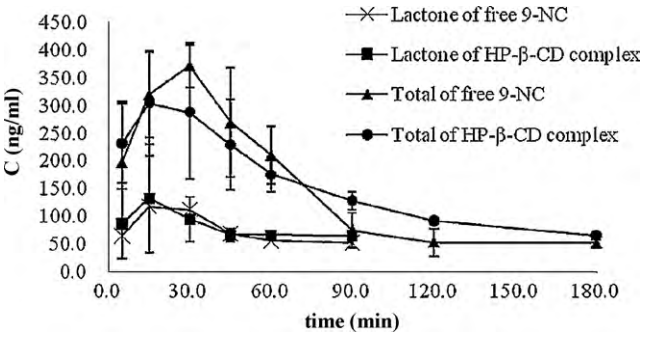

Fig. 7. Plasma concentration–time profile of 9-NC in rats via i.m. ($n = 6$).

3.7. Pharmacokinetics in rat plasma

The HPLC method for analysis has been validated. Linearity in the standard curves was demonstrated over the concentration range studied. The limit of detection (LOD) for 9-NC defined as a minimum signal-to-noise of three was 0.3 ng. The intra-day and the inter-day precision (R.S.D.) was less than 5%. The recovery was 97.23–112.29%.

Injection is the traditional route in cancer therapy. It was found that the injection routes have significant influence on pharmacokinetics and lactone/carboxylate equilibrium of 9-NC (Chen et al., 2007). In this study, free 9-NC and 9-NC/HP-β-CD complex solution were compared via i.m. and i.v. administration. The mean plasma concentration-time profiles of lactone and total 9-NC after i.m. or i.v. administration of free 9-NC or complex solution are shown in Figs. 7 and 8, respectively. The pharmacokinetic parameters are summarized in Tables 5 and 6.

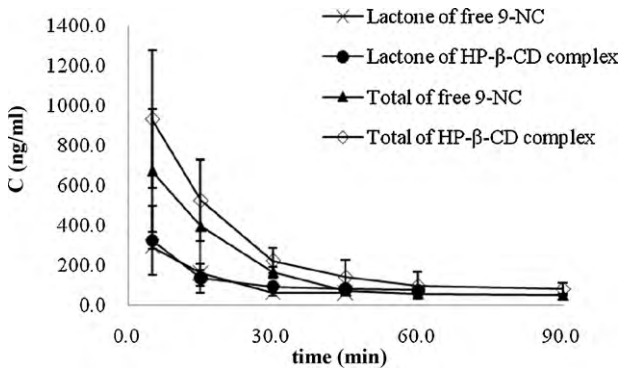

Fig. 8. Plasma concentration–time profile of 9-NC in rats via i.v. ($n = 6$).

Table 5
Mean pharmacokinetic parameters after i.m. administration of free 9-NC and 9-NC/HP-β-CD complex solution ($n = 6$).

Parameter	Free 9-NC	Complex
$t_{1/2}$ (h)	0.44 ± 0.05	0.95 ± 0.08*
Ke (h^{-1})	1.58 ± 0.19	0.73 ± 0.09*
$AUC_{0-3\,h}$ (ng h/ml)	401.42 ± 49.91	561.32 ± 57.32*
$AUC_{0-1.5\,h}$ (ng h/ml) of lactone form	108.96 ± 5.68	146.64 ± 40.91
$AUC_{0-\infty}$ (ng h/ml)	439.39 ± 56.70	632.79 ± 35.1*
$AUC_{0-\infty}$ (ng h/ml) of lactone form	153.72 ± 8.15	260.14 ± 34.99*
C_{max} (ng/ml)	420.44 ± 92.91	285.93 ± 21.78
t_{max} (min)	0.50 ± 0.00	0.33 ± 0.14

Control: free 9-NC.
* $P < 0.05$.

Table 6
Mean pharmacokinetic parameters after i.v. administration of free 9-NC and 9-NC/HP-β-CD complex solution ($n = 6$).

Parameter	Free 9-NC	Complex
$t_{1/2}$ (h)	0.32 ± 0.14	0.34 ± 0.15
Ke (h^{-1})	2.52 ± 0.96	2.3 ± 0.78
V (L/kg)	1.92 ± 0.47	0.51 ± 0.50*
$C_{5\,min}$ (ng/ml)	653.0 ± 238.9	1021.0 ± 362.0*
$AUC_{0-1.5\,h}$ (ng h/ml)	302.2 ± 95.5	419.5 ± 105.9
$AUC_{0-1\,h}$ (ng h/ml) of lactone form	125.9 ± 12.8	142.9 ± 22.0
$AUC_{0-\infty}$ (ng h/ml)	385.39 ± 127.32	538.05 ± 107.29*
$AUC_{0-\infty}$ (ng h/ml) of lactone form	181.38 ± 17.5	272.04 ± 41.6*

Control: free 9-NC.
* $P < 0.05$.

Compared to free 9-NC, $t_{1/2(Ke)}$ of the complex was significantly prolonged (0.95 ± 0.08 vs. 0.44 ± 0.05 h, $P < 0.05$). It was probably the complex was slowly absorbed into the blood circulation and eliminated slowly as well. The slow absorption of 9-NC contributed to a lower C_{max} of complex than that of free 9-NC (285.93 ± 21.78 ng h/ml vs. 420.44 ± 2.91 ng h/ml for complex and free 9-NC, respectively, $P < 0.05$) and the slow elimination resulted in the increased area under the plasma concentration vs. time curve.

It was also found that after i.v. administration, $AUC_{0-\infty}$ was dramatically higher for complex than that of free 9-NC (538.05 ± 107.29 ng h/ml vs. 385.39 ± 127.32 ng h/ml for complex and free 9-NC, respectively, $P < 0.05$). Cylodextrin decreased the distribution of 9-NC to tissues due to its hydrophilic character, and therefore, the drug concentration of the complex in the plasma was higher than that of the free 9-NC. However, complexation with cyclodextrin did not prolong $t_{1/2}$. Both the free 9-NC and complex were eliminated rapidly from the blood plasma.

It is worth noting that for the complex, compared to free 9-NC, its $AUC_{0-\infty}$ of the lactone form, namely the main active form, increased dramatically along with the $AUC_{0-\infty}$ of total 9-NC (260.14 ng h/ml vs. 153.72 ng h/ml for i.m. administration and 272.04 ng h/ml vs. 181.36 ng h/ml for i.v. administration, respectively). In addition, the lactone/total ratio of 9-NC, for i.m. and i.v. administration, increased from 35.0% to 41.1% and 47.1% to 50.6% after complexation with HP-β-CD. This probably occurs because the complexed form of 9-NC, which is in equilibrium with the free 9-NC in blood, is less prone to hydrolysis. This suggested that the complex may have better pharmacodynamic performance. In order to study the therapeutic efficacy of the complex, the in vitro cytotoxicity and in vivo anti-tumor activity are under way.

4. Conclusion

We have developed a HP-β-CD-based formulation with high solubility and stability for 9-NC using a unique but simple and highly reproducible method. Through formulation with HP-β-CD,

the solubility of the extremely hydrophobic compound 9-NC was improved dramatically. Furthermore, the freeze-dried product of the complex had excellent re-dissolution ability and displayed better dissolution performance in comparison with free drug. Pharmacokinetic studies of 9-NC formulations in rats indicated that the complex had higher bioavailability and ratio of lactone form in plasma compared to free 9-NC, which suggested that the complex may exhibit better therapeutic efficacy. Compared to previous 9-NC formulations studies in our lab, such as 9-NC loaded liposomes and solid lipid nanoparticles, HP-β-CD-based delivery system is simple to prepare and presents little or no concerns of instability during storage.

Acknowledgement

This study was supported by the National Science and Technology Major Project (2009ZX09310-006) in China.

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