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Solubilization and preformulation of poorly water soluble and hydrolysis susceptible *N*-epoxymethyl-1,8-naphthalimide (ENA) compound

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Abstract

N-Epoxymethyl-1,8-naphthalimide (ENA) is a novel antiproliferative drug candidate with potent anticancer and antifungal activity. It has an aqueous solubility of 0.0116 mg/mL and also exhibits hydrolytic instability with a first-order hydrolysis rate of 0.051 h^{-1}. The present preformulation study aimed to characterize the physicochemical properties of ENA and develop an early injectable solution formulation for preclinical studies. To minimize hydrolysis, ENA is proposed to be formulated as either lyophilized powders or nonaqueous solutions followed by solubilization/reconstitution prior to administration. ENA solubilization was investigated in both aqueous media (by cosolvency, micellization and complexation) and nonaqueous solutions (mixture of Cremophor EL and ethanol). It is found that none of the solubilization techniques in aqueous media could increase ENA solubility to a desired level of several hundreds μg/mL at pharmaceutically acceptable excipient concentrations (≤10%). In contrast, a combination of 70% Cremophor EL and 30% ethanol (v/v) proved effective in solubilizing ENA at 4 mg/mL, which exhibited good physical and chemical stability on storage at both 4 °C and room temperature over 4 months. No precipitation was observed upon 5–20 times dilution by the saline; in addition, less than 5% of ENA was hydrolyzed in 4 h for the saline-diluted aqueous solutions. This nonaqueous ENA formulation is thus proposed for further preclinical studies, which can be reconstituted, prior to administration, by the 5–20 times infusion fluids (saline, 5% dextrose, etc.) to the desired drug dosing concentration at the acceptable excipient level. The approach used in this work could serve as a useful reference in formulating nonpolar drugs with hydrolytic instability.

Keywords: *N*-Epoxymethyl-1,8-naphthalimide (ENA); Solubilization; Early formulation; Preformulation; Hydrolytic instability

1. Introduction

ENA (*N*-epoxymethyl-1,8-naphthalimide, also known as ENI, Fig. 1) has recently been reported as a novel synthetic antiproliferative agent. By inducing the S phase arrest in the cell cycle, ENA has been found to possess potent anticancer and antifungal activity (Surana et al., 2006; Krishnan et al., 2007). However, ENA is poorly soluble in water. Its aqueous solubility is measured to be 0.0116 mg/mL, which is categorized as practically insoluble (Liu, 2000). In addition, the ENA molecule is found prone to hydrolytic degradation in practice causing decreased antiproliferative activity. Due to these two reasons, it is difficult to formulate an early injectable aqueous solution. The difficulty is worsened by the availability of the expensive drug candidate only in limited quantities and a tight developmental timeline. The purpose of the current preformulation study is thus to determine the physicochemical properties of ENA and to develop an early injectable solution formulation for further preclinical investigations.

Early formulation development exists as an integral part in drug discovery and development (Li and Zhao, 2007). Developing an early intravenous aqueous solution formulation for

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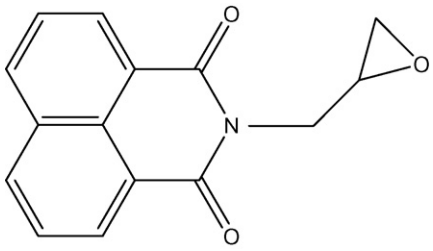

Fig. 1. Chemical structure of ENA.

hydrolytic unstable nonpolar drugs remains a challenge as both enhancement of solubility and minimization of chemical degradation needs to be addressed. To enhance the aqueous solubility of poorly water-soluble drugs, the commonly used techniques in marketed products include pH adjustment, cosolvency, micellization and complexation, which corresponds to the addition of buffers, cosolvents (ethanol, glycerol, propylene glycol, PEG400, etc.), surfactants (Tween 80, Cremophor EL, etc.) and complexants (mainly various types of cyclodextrin) to aid solubilization (Yalkowsky, 1999; Strickley, 2004; Li and Zhao, 2007). Combination of two or more of these techniques may produce synergistic effect in solubilization (Li et al., 1999; Ni et al., 2002a; Chang and Shojaei, 2004; Yang et al., 2004; Kawakami et al., 2006). Other techniques, such as polymeric micelles (Barreiro-Iglesias et al., 2004), emulsions and liposomes (Li and Zhao, 2007) have also been reported in the literature. On the other hand, to maintain chemical stability of hydrolytic unstable drugs, removing or minimizing the contact with water in the formulation is a simple but effective strategy. The commonly used approach in the pharmaceutical industry is to formulate hydrolytic unstable drugs as either: (1) lyophilized powders, which is solubilized in the aqueous medium by the aforementioned techniques followed by reconstitution with infusion fluids (saline, 5% dextrose, etc.) prior to administration, or (2) semi-aqueous or nonaqueous solutions prepared by dissolving the drug in pharmaceutically acceptable organic solvents, surfactants (e.g. Tween 80, Cremophor EL, etc.) or their combinations (Zhao and Yalkowsky, 2001; Ni et al., 2002b; Zhu et al., 2002). The obtained semi-aqueous or nonaqueous solution formulation is reconstituted just before intravenous administration. Both the lyophilized powder and semi/non-aqueous solution formulation is able to avoid or minimize the degradation of the hydrolysis susceptible drug by shortening the contact time with water before administration. Two examples of such commercial formulations are melphalan (Alkeran®) and Carmustine (BiCNU®). Formulating hydrolytic unstable drugs in cyclodextrins (Loftsson and Brewster, 1996; Ma et al., 1999) or polymeric micelles (Barreiro-Iglesias et al., 2004) has also been reported to be able to maintain the drug stability.

In this work, to minimize hydrolysis, ENA is proposed to be formulated as lyophilized powders or nonaqueous solutions, since these two kinds of formulations are most commonly used in the pharmaceutical industry, which facilitates further preclinical investigations (Strickley, 2004). Numerous preformulation studies have been performed on the hydrolytic stable nonpolar drugs (solubilization) or hydrolytic unstable water-soluble drugs (stabilization) (Zhao and Yalkowsky, 2001; Ni et al., 2002b; Zhu et al., 2002). However, to our knowledge, few publications have described the preformulation of a drug molecule possessing water insolubility and hydrolytic instability simultaneously (Barreiro-Iglesias et al., 2004; Cappello et al., 2006; Rouf et al., 2007). Solubilities of ENA were studied both in aqueous (in the presence of cosolvents, surfactants or complexing agents) and nonaqueous (the mixture of Cremophor EL and ethanol at different ratio) media for the selection of a suitable preformulation. Degradation (hydrolysis) of ENA was monitored using HPLC by comparing the drug peak area at specific time t and the initial drug peak area ($t = 0$). Physical stability, i.e. the absence of precipitation of the ENA formulation upon storage and in saline-diluted formulation was investigated by visual observation.

2. Materials and methods

2.1. Materials

ENA was kindly provided by the Institute of Molecular and Cell Biology of Singapore. The cosolvents ethanol and polyethylene glycol 400 (PEG400) were purchased from VWR International Ltd. and Merck, respectively; while propylene glycol (PG) and glycerol was from Alfa Aesar. HPLC grade acetonitrile was supplied by Merck. The surfactants Tween 80 and Cremophor EL were obtained from Sigma. The complexants hydroxypropyl-β-cyclodextrin (HPβCD) and hydroxypropyl-γ-cyclodextrin (HPγCD) were from Acros Organics and Sigma, respectively.

2.2. Characterization of ENA

Morphology of ENA particles was observed by a field emission scanning electron microscope (FESEM, JEOL JSM-6700F). Before visualization, the drug particles were coated with gold for 40 s. Thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC) were conducted on Diamond DSC Calorimeter (PerkinElmer) and Simultaneous DSC–TGA (STD2960, TA Instruments), respectively. During both measurements, the samples were heated at 10 °C/min in nitrogen atmosphere. X-ray diffraction measurements were performed on a D8-ADVANCE (BRUKER) X-ray diffractometer in steps of 0.02° using Cu Kα radiation.

2.3. Solubility measurement

The solubility of ENA in aqueous or nonaqueous solutions was determined by the Higuchi and Connor method (Higuchi and Connors, 1965). In brief, excess amount of ENA was added to the 2 mL aqueous (with cosolvents, surfactants or complexants at different concentrations) or nonaqueous media in a capped vial and stirred for 48 h to reach equilibrium. The resultant suspension was filtered through 0.45 μm (pore size) filter. The filtrate was appropriately diluted for HPLC analysis. All the experiments were done in duplicate.

Fig. 2. FESEM image of ENA particles.

2.4. Degradation kinetic studies in water

Excess amount of ENA was suspended in 2 mL water in a capped vial. After 2 and 48 h of stirring, respectively, the suspension was filtered through 0.45 μm (pore size) filter and the filtrate was used directly for HPLC analysis at specific times at room temperature. Degradation (hydrolysis) of ENA was monitored using HPLC by comparing the drug peak area at specific time t and the initial drug peak area ($t = 0$).

2.5. HPLC assay

ENA content was quantitatively analyzed by the HPLC (Agilent 1100) equipped with the Agilent Eclipse XDB-C18 column (5 μm, 4.6 mm × 250 mm). The mobile phase, composed of 50% acetonitrile and 50% water, was delivered at 1 mL/min. ENA was detected at 336 nm. Presence of cosolvents, surfactants, or complexing agents has no interference with the assay. The ENA calibration curve was linear in the range of 1–100 μg/mL.

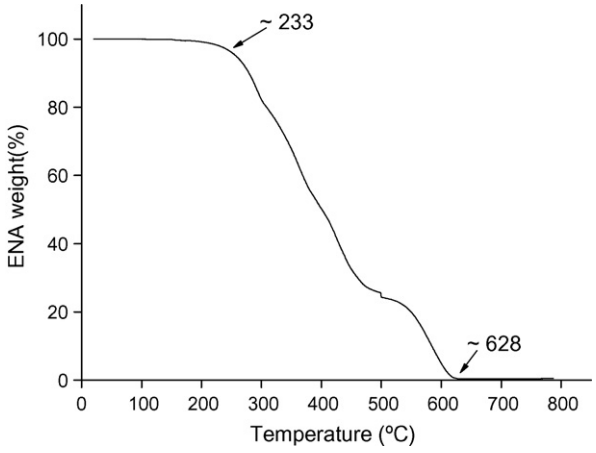

Fig. 3. TGA of ENA.

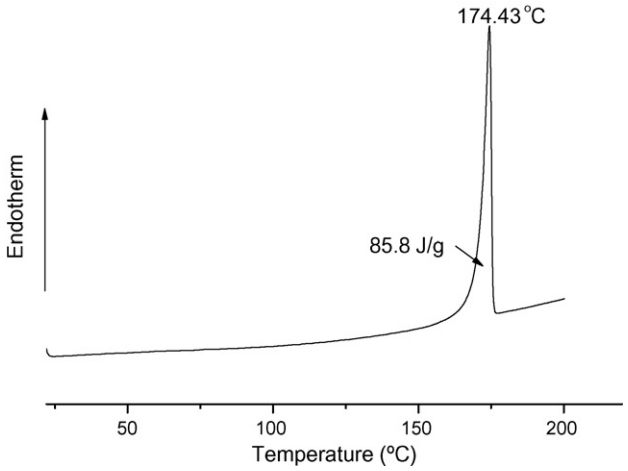

Fig. 4. DSC of ENA.

3. Results and discussion

3.1. Preformulation characterization of ENA

As shown in Fig. 1, ENA is a derivative of 1,8-naphthalimide with the hydrogen atom of the imide backbone substituted by the epoxymethyl side group with a molecular weight of 253.25. SEM image (Fig. 2) revealed a relatively distinct morphology with well-defined edges and also a broad particle size distribution. TGA analysis shown in Fig. 3 indicated that ENA was thermally stable up to 233 °C before decomposition sets in. The melting point and enthalpy of fusion were determined to be 174.4 °C and 85.8 J/g as shown in the DSC thermogram (Fig. 4). The calculated log P of ENA was 1.23 ± 0.64 (by ADC\chemsketc). Owing to the relatively high melting point, bulky molecular structure and the lack of ionizable groups, it is unsurprising that ENA is practically insoluble in water and its aqueous solubility was determined to be 0.0116 mg/mL. PXRD diffractogram in Fig. 5 shows relatively distinct intensity peaks, which indicates a predominantly crystalline compound.

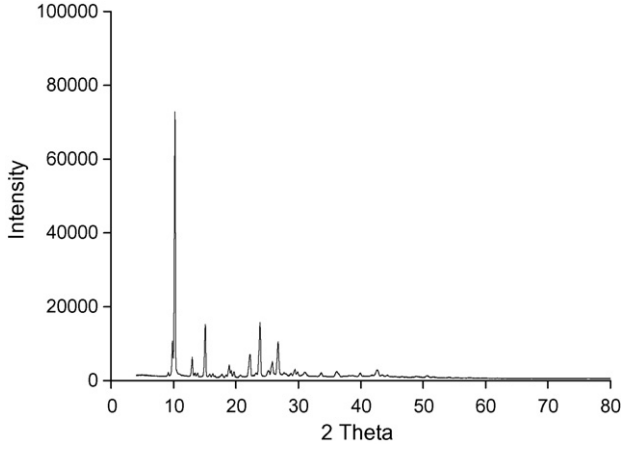

Fig. 5. PXRD of ENA.

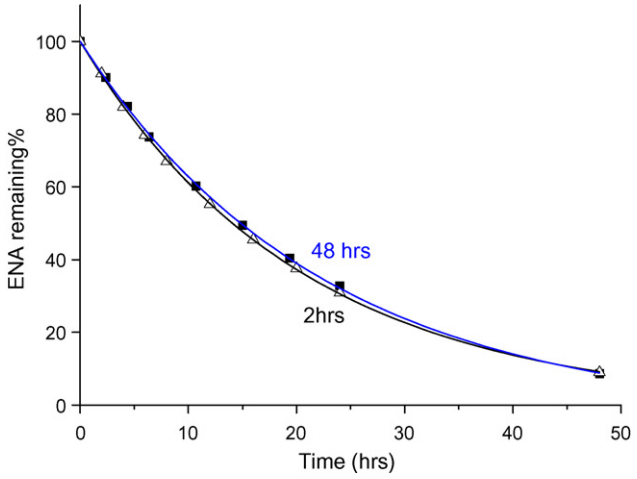

Fig. 6. Degradation profile of ENA in water.

3.2. Degradation kinetics of ENA

Due to the instability of the epoxy ring and/or imide group, ENA molecule is susceptible to hydrolysis in aqueous media. The degradation curves of ENA aqueous solutions, obtained from 2 to 48 h' solubilization, respectively, are shown in Fig. 6. It can be seen that degradation of ENA follows the apparent first-order degradation kinetics and there is no difference between the two ENA solutions. From the slope of the logarithmic percentage of remaining ENA vs. time curve, the degradation rate of ENA was determined to be 0.051 h^{-1}. After 72 h, no ENA was detected showing that the degradation process was irreversible.

3.3. Solubility of ENA in aqueous solution

To solubilize poorly water-soluble drugs in aqueous media, techniques often adopted in the pharmaceutical industry include pH adjustment, cosolvency, micellization and complexation. Since ENA is non-ionizable, pH adjustment is thus not investigated. Solubility measurements in water showed that the saturated solution concentration of ENA was obtained after 48 h, which remained constant upon subsequent prolonged stirring. Therefore, the duration of 48 h was selected for all solubility measurements. It was also found that ENA exhibits a relatively high dissolution rate, whereby the ENA solution concentration reaches 90% of the saturated solution concentration within 2 h. As the degradation of ENA is irreversible, the following reactions would occur in an aqueous medium:

$$\text{solid drug powder} \leftrightarrow \text{solubilized drug(measured solubility)}$$

$$\rightarrow \text{degraded drug.}$$

In this system, since the rate of dissolution of ENA is fast in comparison with its degradation, the measured ENA solubility thus remains constant as long as the solid ENA exists.

3.3.1. Cosolvency

Cosolvency is extensively used to solubilize the water-insoluble drugs due to its effectiveness and simplicity. Cosolvents are water miscible organic compounds having small hydrocarbon regions. Compared to water, the cosolvent-water system has increased nonpolarity and reduced ability to squeeze out the nonpolar molecules (Ran et al., 2001). Water insoluble drugs are thus solubilized in the cosolvent-water system. Fig. 7 shows the solubilization curve of ENA by the cosolvency technique. The cosolvents used were ethanol, PEG400, PG and glycerol, which are widely used in marketed parenteral formulations. The solubility of ENA in water (S_0) was measured to be 0.0116 mg/mL. For all the cosolvent-water systems studied, the solubility of ENA was increased exponentially with the increase of cosolvent concentration. 50% of ethanol, PEG400, PG and glycerol was found to increase the solubility of ENA from 0.0116 to 0.5314, 0.2691, 0.1815 and 0.0383 mg/mL, respectively. The relationship between the logarithmic drug solubility in the cosolvent-water system (S) and the cosolvent concentration ($C_{\text{cosolvent}}$) can be described by the following equation (Yalkowsky, 1999; He et al., 2005):

$$\log S = \log S_0 + \sigma C_{\text{cosolvent}},$$

σ : cosolvent solubilization power

The solubilization power, σ, for ethanol, PEG400, PG and glycerol was thus determined from the slope of the fitted $\log S$ vs. $C_{\text{cosolvent}}$ curve, to be 0.0344, 0.0266, 0.0235 and 0.0106%$^{-1}$. Ethanol is most effective to solubilize ENA followed by PEG400, PG and glycerol. This solubilization power order follows the rule that the less polar the cosolvent, the more effective the cosolvent-water system to solubilize the nonpolar drug (Zhao et al., 1999).

3.3.2. Micellization

Surfactants are amphiphilic molecules, which are extensively used in drug formulations for solubility and stability enhancement. In aqueous medium, when the surfactant concentration is above its critical micellar concentration (CMC), micelles are formed by assembling the hydrophobic segments of the surfactant molecules to form the nonpolar core while their hydrophilic segments stretching out to form the shell. Water insoluble drugs are solubilized by their incorporation

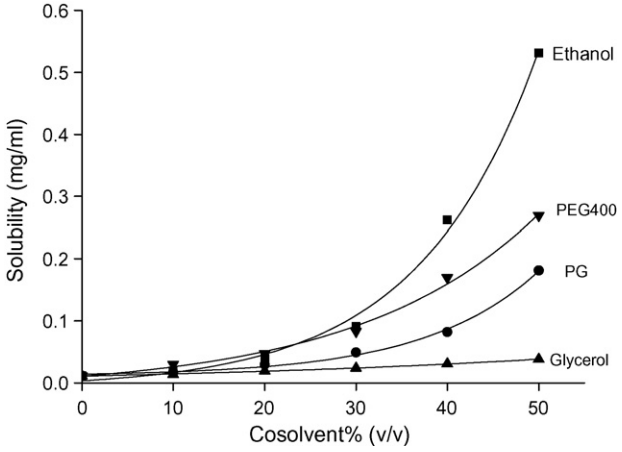

Fig. 7. Solubilization of ENA by cosolvency.

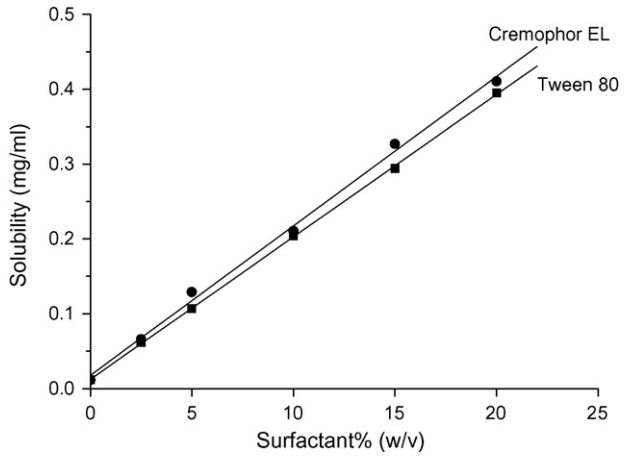

Fig. 8. Solubilization of ENA by micellization.

into the nonpolar inner core of the micelles. Tween 80 and Cremophor EL are the two commonly used surfactants to formulate drugs due to their potent solubilization capacity. As seen from Fig. 8, the solubility of ENA was increased linearly with the increase of surfactant concentration both for Tween 80 and Cremophor EL. 20% of Tween 80 and Cremophor EL increased the solubility of ENA from 0.0116 to 0.3950 and 0.4104 mg/mL, respectively. The relationship between the drug solubility in the presence of surfactants (S) and the surfactant concentration (C_{surf}) can be approximated as (Yalkowsky, 1999; He et al., 2005; He and Yalkowsky, 2006):

$$S \approx S_0 + \kappa\, C_{surf}, \quad \kappa : \text{surfactant solubilization power}$$

The solubilization power, κ, determined from the slope of the solubilization curve, was 0.0199 mg/mL for Cremophor EL and 0.0190 mg/mL for Tween 80. These two surfactants possessed quite similar capacity to solubilize ENA.

3.3.3. Complexation

Cyclodextrins are cyclic oligosaccharides with a nonpolar central cavity, which is composed by the 6 (α-CD), 7 (β-CD) or 8 (γ-CD) glucose units. In aqueous solution, the water insoluble compound is able to be solubilized by fitting into the cavity of the cyclodextrin to form the water soluble noncovalent inclusion complex (Ma et al., 1999; Moraes et al., 2007; Loftsson and Duchêne, 2007). HPβCD and HPγCD were used in our study due to their extensive application in the drug formulations. Fig. 9 shows the solubilization profile of ENA by complexation with cyclodextrin. It is obvious that, whether for HPβCD or HPγCD, the solubility of ENA is increased linearly with the cyclodextrin concentration (A_L type) indicating the formation of a 1:1 ENA-cyclodextrin complex. 20% of HPβCD and HPγCD were found to increase the solubility of ENA from 0.0116 to 0.1697 and 0.1902 mg/mL, respectively. For the 1:1 drug-cyclodextrin complex, the relationship between the drug solubility (S) and the cyclodextrin concentration ($C_{complex}$) is expressed by the following equation (He and Yalkowsky, 2006):

$$S \approx S_0 + \tau\, C_{complex}, \quad \tau : \text{solubilization power}$$

$$K^{1:1} = \frac{\tau}{S_0}, \quad K^{1:1} : \text{complexation constant}$$

The solubilization power was calculated to be 0.0078 and 0.0086 mg/mL for HPβCD and HPγCD, respectively. The complexation constant was 93.77 and 116.83 M^{-1} for ENA-HPβCD and ENA-HPγCD complex, respectively if both the solubility of ENA and the concentration of cyclodextrin are expressed in molar concentration (M).

From the above results, it can be seen that, cosolvency, micellization and complexation does improve the aqueous solubility of ENA. However, the extent of solubility enhancement by either of them is not as pronounced as some other poorly water-soluble drugs. The reason for the low effectiveness of solubilization by these techniques could be attributed to the relatively low nonpolarity of ENA indicated by its log P (1.23 ± 0.64). It is known that polarity, geometry, conformation, and intermolecular interactions of a drug have significant influence on its solubilization effectiveness in aqueous media (Ran et al., 2001; He et al., 2005). The relatively low nonpolarity of ENA results in a weak hydrophobic interaction with the nonpolar regions of the cosolvents (cosolvency), nonpolar core of the micelles (micellization) or nonpolar cavity of the cyclodextrins (complexation) leading to a low solubilization effectiveness.

3.4. Solubility in nonaqueous solution

Formulating water-insoluble compounds in nonaqueous media is not one of the first-choices, but it is quite effective to solubilize some challenging drugs (Strickley, 2004). The combination of Cremophor EL (solubilizer) and ethanol (thinning agent and cosolvent) is commonly employed to solubilize poorly water-soluble drugs in practice, such as Valstar™ Medeva (40 mg/mL valrubicin in 50% Cremophor EL and 50% ethanol), Taxol® (6 mg/mL paclitaxel in 51% Cremophor EL and 49% ethanol) and Sandimmune® (50 mg/mL cyclosporin A in 67% Cremophor EL and 33% ethanol). In this work, combination of Cremophor EL and ethanol in different ratios was

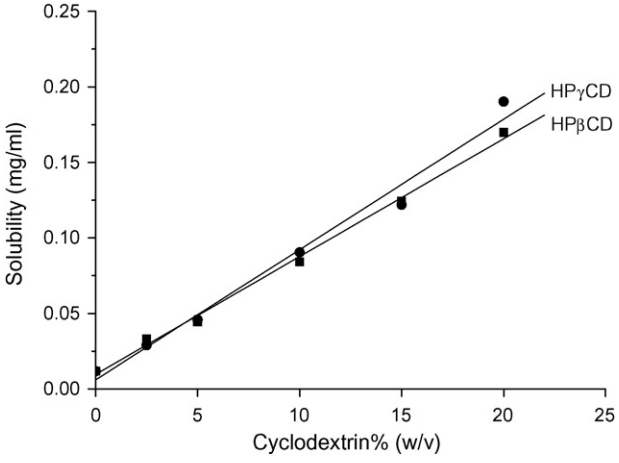

Fig. 9. Solubilization by complexation.

Table 1
Solubility of ENA in the mixture of Cremophor EL and ethanol

Cremophor EL:ethanol (v/v)	Solubility (mg/mL)
0%:100%	1.25
50%:50%	3.90
60%:40%	4.54
70%:30%	5.42
100%:0%	6.65

investigated to formulate ENA for both solubility and stability purpose. An optimized ratio of Cremophor EL to ethanol results from a trade-off between increase in solubility and ease of handling, since increasing Cremophor EL content leads to the formation of a highly viscous gel and subsequent handling problems as previously reported (Cuine et al., 2007). As shown in Table 1, the solubilities of ENA in the mixture of Cremophor EL and ethanol at v/v ratios 0%:100%, 50%:50%, 60%:40%, 70%:30%, 100%:0% were 1.25, 3.90, 4.54, 5.42 and 6.65 mg/mL, respectively, which represents 108, 335, 390, 465 and 573 times increase in solubility.

3.5. Selection of the ENA early injectable formulation

To produce the therapeutic efficacy, the dosing concentration of the intravenously administered drug should be maintained at some specific level, which is found to be several hundreds μg/mL for most of the drugs (Strickley, 2004). Meanwhile, to avoid the excipient-caused side effects, the concentration of the excipient also has an upper limit, which is generally 10% although higher excipient concentration is also used in some cases (Zhao et al., 1999; Strickley, 2004). From the solubility data of ENA in aqueous solution, it is obvious that, none of the cosolvency, micellization or complexation is able to enhance the ENA solubility to several hundreds μg/mL at the excipient concentration of 10%. Therefore, preformulation of ENA as lyophilized powder followed by solubilization prior to intravenous administration is precluded. In contrast, the mixture of Cremophor EL and ethanol seems quite effective to solubilize ENA.

A suitable solution intravenous formulation generally meets the following requirements: (1) the drug concentration is high for convenient storage, (2) the formulation is chemically and physically stable on storage and (3) there is no precipitation when reconstituted with infusion fluids and after intravenously administered to the blood. Based on these principles, four formulations were thus prepared: (A) 4 mg/mL ENA in the mixture of 60% Cremophor EL and 40% ethanol (v/v), (B) 4 mg/mL ENA in the mixture of 70% Cremophor EL and 30% ethanol (v/v), (C) 4.5 mg/mL ENA in the mixture of 70% Cremophor EL and 30% ethanol (v/v) and (D) 5 mg/mL ENA in the mixture of 70% Cremophor EL and 30% ethanol (v/v). These formulations were then 5, 10, 15 and 20 times diluted by the saline. It was found that, only for formulation B, i.e. 4 mg/mL ENA in the mixture of 70% Cremophor EL and 30% ethanol, no precipitation was observed upon dilution in 4 h. In addition, less than 5% of the ENA was hydrolyzed in 4 h for the 5–20 times saline-diluted aqueous solution. Such a formulation is chemically and physically stable whether stored at 4 °C or room temperature for 4 months. Formulation of 4 mg/mL ENA in the mixture of 70% Cremophor EL and 30% ethanol is thus proposed for further preclinical investigations.

4. Conclusion

Preformulation characterization and solubilization of ENA were conducted in this work to find a suitable early injectable formulation for further preclinical studies. Based on the solubility of ENA in aqueous (by cosolvency, micellization and complexation) and nonaqueous (mixture of Cremophor EL and ethanol with different ratio) solutions and their physicochemical stability, formulation of 4 mg/mL ENA in a mixture of Cremophor EL and ethanol at 70%:30% (v/v) is proposed, which is physically and chemically stable over 4 months upon storage at room temperature or 4 °C. Prior to administration, the formulation can be diluted 5–20 times with infusion fluids without precipitation to get the drug concentration of several hundreds μg/mL at the allowable excipient level (≤10%). After dilution, less than 5% of ENA was found to be hydrolyzed within 4 h. This work provides an added reference to the development of early injectable formulations of drugs, which have both poorly water-soluble and hydrolysis sensitive properties.

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References

Barreiro-Iglesias, R., Bromberg, L., Temchenko, M., Hatton, T.A., Concheiro, A., Alvarez-Lorenzo, C., 2004. Solubilization and stabilization of camptothecin in micellar solutions of pluronic-g-poly(acrylic acid) copolymers. J. Control. Release 97, 537–549.

Cappello, B., Di Maio, C., Iervolino, M., Miro, A., 2006. Improvement of solubility and stability of valsartan by hydroxypropyl-beta-cyclodextrin. J. Incl. Phenom. Macro. 54, 289–294.

Chang, R.-K., Shojaei, A.H., 2004. Effect of hydroxypropyl β-cyclodextrin on drug solubility in water-propylene glycol mixtures. Drug Dev. Ind. Pharm. 30, 297–302.

Cuine, J.F., Charman, W.N., Pouton, C.W., Edwards, G.A., Porter, C.J.H., 2007. Increasing the proportional content of surfactant (cremophor EL) relative to lipid in self-emulsifying lipid-based formulations of danazol reduces oral bioavailability in beagle dogs. Pharm. Res. 24, 748–757.

He, Y., Tabibi, S.E., Yalkowsky, S.H., 2005. Solubilization of two structurally related anticancer drugs: XK-469 and PPA. J. Pharm. Sci. 95, 97–107.

He, Y., Yalkowsky, S.H., 2006. Solubilization of monovalent weak electrolytes by micellization or complexation. Int. J. Pharm. 314, 15–20.

Higuchi, T., Connors, K.A., 1965. Phase-solubility techniques. Adv. Anal. Chem. Inst. 4, 117–212.

Kawakami, K., Oda, N., Miyoshi, K., Funaki, T., Ida, Y., 2006. Solubilization behavior of a poorly soluble drug under combined use of surfactants and cosolvents. Eur. J. Pharm. Sci. 28, 7–14.

Krishnan, V., Dirick, L., Lim, H.H., Lim, T.S.J., Si-Hoe, S.L., Cheng, C.S., Yap, K.L., Ting, A., Schwob, E., Surana, U., 2007. A novel cell cycle

inhibitor stalls replication forks and activates S phase checkpoint. Cell Cycle 6, 1621–1630.

Li, P., Zhao, L., Yalkowsky, S.H., 1999. Combined effect of cosolvent and cyclodextrin on solubilization of nonpolar drugs. J. Pharm. Sci. 88, 1107–1111.

Li, P., Zhao, L., 2007. Developing early formulations: practice and perspective. Int. J. Pharm. 341, 1–19.

Liu, R., 2000. Water-insoluble Drug Formulation, 1st ed. CRC Press.

Loftsson, T., Brewster, M.E., 1996. Pharmaceutical application of cyclodextrins. 1. Drug solubilization and stabilization. J. Pharm. Sci. 85, 1017–1025.

Loftsson, T., Duchêne, D., 2007. Cyclodextrins and their pharmaceutical applications. Int. J. Pharm. 329, 1–11.

Ma, D.Q., Rajewski, R.A., Stella, V.J., 1999. New injectable melphalan formulations utilizing $(SBE)_{7m}$-β-CD or HP-β-CD. Int. J. Pharm. 189, 227–234.

Moraes, C.M., Abrami, P., Paula, E.D., Braga, A.F.A., Fraceto, L.F., 2007. Study of the interaction between S(−) bupivacaine and 2-hydroxypropyl-β-cyclodextrin. Int. J. Pharm. 331, 99–106.

Ni, N., Sanghvi, T., Yalkowsky, S.H., 2002a. Solubilization and preformulation of carbendazim. Int. J. Pharm. 244, 99–104.

Ni, N., Sanghvi, T., Yalkowsky, S.H., 2002b. Stabilization and preformulation of anticancer drug-SarCNU. Int. J. Pharm. 249, 257–264.

Ran, Y., Zhao, L., Xu, Q., Yalkowsky, S.H., 2001. Solubilization of cyclosporin A. AAPS Pharmscitech. 2 (article 2).

Rouf, M.A., Bilensoy, E., Vural, I., Hincal, A.A., 2007. Inclusion complexation of rapamycin with beta-cyclodextrin to improve solubility and stability of the drug. Eur. J. Pharm. Sci. 32, S46–S47.

Strickley, R.G., 2004. Solubilizing excipients in oral and injectable formulations. Pharm. Res. 21, 201–230.

Surana, U., Krishnan, V., Ting, A., Lim, H.H., 2006. Naphthalimide derivatives having antiproliferative activity. WO/2006/104461.

Yalkowsky, S.H., 1999. Solubility and Solubilization in Aqueous Media. Oxford University Press, New York.

Yang, G., Jain, N., Yalkowsky, S.H., 2004. Combined effect of SLS and (SBE)7M-β-CD on the solubilization of NSC-639829. Int. J. Pharm. 269, 141–148.

Zhao, L., Li, P., Yalkowsky, S.H., 1999. Solubilization of fluasterone. J. Pharm. Sci. 88, 967–969.

Zhao, L., Yalkowsky, S.H., 2001. Stabilization of eptifibatide by cosolvents. Int. J. Pharm. 218, 43–56.

Zhu, H., Meserve, K., Floyd, A., 2002. Preformulation studies for an ultrashort-acting neuromuscular blocking agent GW280430A. I. Buffer and cosolvent effects on the solution stability. Drug Dev. Ind. Pharm. 28, 135–142.