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ORIGINAL ARTICLE

Solubilization of vorinostat by cyclodextrins

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SUMMARY

Background: **Vorinostat (suberoylanilide hydroxamic acid) is the first histone deacetylase inhibitor approved by US FDA for use in oncology. However, as a hydrophobic acid, its limited aqueous solubility poses a problem for parenteral delivery. Such limited solubility may also affect its oral bioavailability.**

Objective: **The aim of this study was to evaluate whether cyclodextrins (CDs), common excipients used in pharmaceutical industry, could increase the aqueous solubility of vorinostat.**

Methods: **The actual aqueous solubility of vorinostat was investigated by phase-solubility method. Molecular simulation was employed to predict the interaction energy and preferred orientation of vorinostat in CD cavities.**

Results: **Phase-solubility studies indicated that the solubility of vorinostat ($7{\cdot}24 \times 10^{-1}$ mM) was substantially increased when complexed with various CDs, in the following order: randomly methylated-β-cyclodextrin (RM-β-CD) > hydroxypropyl-β-cyclodextrin (HP-β-CD) > α-cyclodextrin > hydroxypropyl-α-cyclodextrin > Hydroxypropyl-γ-cyclodextrin > γ-cyclodextrin. RM-β-CD 300 mM increased vorinostat solubility to 70·8 mM, almost two orders of magnitude higher than the baseline solubility. Such findings were in good agreement with the results obtained from molecular simulation.**

Conclusion: **CDs, particularly RM-β-CD and HP-β-CD, increased vorinostat's solubility. Future studies could be focused on the application of HP-β-CD in parenteral delivery of vorinostat or using RM-β-CD as an oral absorption enhancer. Molecular simulation appeared to be a useful tool for the selection of appropriate CD as excipient for drug delivery.**

***Keywords*: cyclodextrin, inclusion complex, molecular simulation, phase solubility, vorinostat**

INTRODUCTION

Vorinostat, also known as suberoylanilide hydroxamic acid or *N*-hydroxy-*N*′-phenyloctanediamide (Fig. 1), is a histone deacetylase inhibitor originally developed by Marks and Breslow for its anti-neoplastic effects (1). Vorinostat effectively induces cell cycle arrest, cell differentiation and/or apoptotic cell death in various transformed cells at micromolar level (1–3). The clinical efficacy of vorinostat in oncology had been demonstrated and the drug was approved by US FDA for the treatment of cutaneous T cell lymphoma in October 2006 (1, 4). The applications of vorinostat in other solid or haematological malignancies are currently under extensive clinical investigations (2, 5). Besides anti-neoplastic effects, the anti-inflammatory activity of vorinostat has been documented in preclinical models of various auto-immune disorders, including inflammatory bowel diseases, rheumatoid arthritis and systemic lupus erythematosus (2, 6). Moreover, vorinostat was shown to ameliorate motor deficits in a mouse model of Huntington's disease (7).

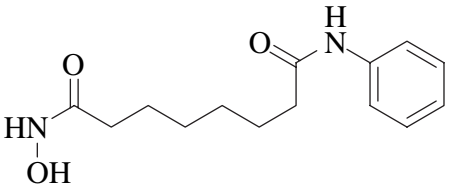

Fig. 1. Molecular structure of vorinostat.

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Vorinostat is a hydrophobic acid with limited aqueous solubility (approximately 0·2 mg/mL), and therefore difficult to formulate for parenteral delivery. In an early phase I clinical trial, vorinostat was administered as a 2-h infusion (8). The intravenous infusion solution was prepared by dissolving vorinostat with sodium hydroxide and adjusting the pH value to 11·2 with hydrochloric acid (8). Such a high pH value makes this formulation unsuitable for routine clinical practice as tedious preparation procedures are required. Moreover, the limited aqueous solubility of vorinostat may also affect its oral bioavailability. Therefore, it is of interest to develop new formulations of vorinostat for both oral and parenteral use.

Cyclodextrins (CDs), cyclic oligosaccharides derived from starch, are well known for their ability to form inclusion complexes with small molecules and portions of large compounds (9). Such inclusion complexes usually offer increased aqueous solubility, improved oral bioavailability and enhanced chemical stability. Moreover, the parenteral safety of hydroxypropyl-β-cyclodextrin (HP-β-CD) had been well documented and HP-β-CD-based intravenous formulations of itraconazole and mitomycin are routinely used in the clinic (9, 10). Therefore, HP-β-CD may be a suitable excipient for parenteral delivery of vorinostat. Furthermore, in a pilot study, Hockly *et al*. solubilized vorinostat with HP-β-CD and supplied it as a therapeutic agent with drinking water to mice with motor deficits (7). However, the phase solubility and physicochemical properties of such inclusion complexes have not been studied. Moreover, randomly methylated-β-cyclodextrin (RM-β-CD), which usually is better at improving aqueous solubility and oral bioavailability of the complexed drug, has never been studied. Therefore, it is hypothesized that both HP-β-CD, RM-β-CD as well as other CD could be appropriate excipients for the delivery of vorinostat. To confirm this hypothesis, phase-solubility and molecular simulation of the inclusion complexes prepared with various CDs were investigated in this study.

MATERIALS AND METHODS

Materials

Vorinostat was purchased from Toronto Research Chemicals (North York, ON, Canada). Hydroxypropyl-γ-cyclodextrin (HP-γ-CD) (degree of substitution: approximately 0·8) was obtained from Research Biochemicals International (Natick, MA, USA). HP-β-CD (degree of substitution: approximately 0·6) was donated by Roquette (Lestrem, France). α-cyclodextrin (α-CD), hydroxypropyl-α-cyclodextrin (HP-α-CD; degree of substitution: approximately 0·5–0·8), RM-β-CD (degree of substitution: approximately 1·8) and γ-cyclodextrin (γ-CD) were generous gifts from Wacker (Burghausen, Germany). Chromatographic grade methanol and acetonitrile were obtained from Tedia (Fairfield, OH, USA). Formic acid was purchased from Fluka Chemika (Buchs, Switzerland). Pure water (18·2 MΩ cm at 25 °C) was generated from a Millipore Direct-Q® ultra-pure water system (Billerica, MA, USA) and used throughout the study.

High performance liquid chromatography (HPLC) assay

High performance liquid chromatography assays were performed for the phase-solubility study. A Shimadzu (Kyoto, Japan) 2010A liquid chromatography was used for the analysis. The separation was performed on a reversed-phase HPLC column (Zorbax Eclipse XDB-C18, 3 × 250 mm, 5 μm; Agilent, Palo Alto, CA, USA), protected by a mechanical filter (Rheodyne, Cotati, CA, USA). The assay was performed at 40 °C through isocratic delivery of the mobile phase, consisting of acetonitrile – 0·1% formic acid (22 : 78, v/v). The flow rate was set at 0·8 mL/min and 10 μL sample was injected into the HPLC system for each run. Vorinostat was quantified by measuring UV absorbance at 241 nM, and calibrated through an external standard method. The calibration curve was linear ($R^2 > 0{\cdot}999$) within the range of 1·0–50·0 μg/mL. The intra-day and inter-day variations were all less than 4%.

Phase-solubility study

The intrinsic aqueous solubility of vorinostat in purified water was first assessed. Vorinostat–CD inclusion complexes were then prepared by a method previously reported (11). An excess amount of vorinostat (20 mg/mL, except 30 mg/mL in RM-β-CD) was added to CD solutions at different concentrations (α-CD: 10, 20, 40, 80, 120 mM; γ-CD and HP-γ-CD: 10, 20, 50, 100, 150 mM; HP-α-CD,

HP-β-CD and RM-β-CD: 10, 20, 30, 50, 100, 200, 300 mM). The suspension was then shaken on a horizontal rotary shaker at a speed of 200 rpm for 2 days. Subsequently, it was filtered through a 0·20-μM syringe filter (Sartorius, Hanover, Germany) to obtain a clear solution. The concentration of vorinostat in inclusion complex solutions was determined by HPLC assay. The apparent inclusion rate constant ($K_{1:1}$) was calculated with the equation established by Higuchi and Connors (12):

$$K_{1:1} = \frac{Slope_1}{S_0 \cdot (1 - Slope_1)};$$

where $Slope_1$ is slope of the phase-solubility curve and S_0 is the solubility of vorinostat in pure water.

Dilution and re-dissolution study of vorinostat-HP-β-CD complex

In order to examine the suitability of HP-β-CD as a parenteral excipient to deliver vorinostat, vorinostat-HP-β-CD solution (10 mg vorinostat was fully dissolved in 1 mL 300 mM HP-β-CD) was diluted with isotonic phosphate buffer (pH = 7·4) to produce 5, 10, 50, 100 and 500 dilutions. The diluted solutions were centrifuged at 10 000 *g* for 10 min and carefully observed for precipitates; 2 mL of vorinostat–HP-β-CD solution (10 mg/mL) was freeze-dried overnight. The dried product was then re-dissolved in isotonic phosphate butter and made up to 2 mL. The re-dissolved solutions were checked for the presence of re-precipitates. Both procedures were carried out in triplicate.

Molecular simulation

A molecular simulation study was performed to study the complexation of vorinostat with the various CDs. Software SYBYL version 7·2 (Tripos Co., St Louis, MO, USA) was used for the study. Dimethyl-β-cyclodextrin (DM-β-CD) was adopted to facilitate determination of stable structure of RM-β-CD as RM-β-CD (degree of substitution = 1·8) is a mixture of different structures. Four 2-hydroxypropyl groups were added on the primary hydroxyl groups of α-CD and β-CD, as shown by Mura *et al.*, to simulate the structure of HP-α-CD (degree of substitution: 0·5–0·8) and HP-β-CD (degree of subtitution: 0·6), respectively (13). The structures of vorinostat, α-CD, HP-α-CD, HP-β-CD, DM-β-CD, γ-CD and HP-γ-CD were individually minimized by MMFF94s force field in the aforementioned software. After minimization, docking experiments were carried out on all molecules using the 'Dock' module in SYBYL. This allowed the vorinostat to move within the energy field of the CDs and vice versa, in order to find the most preferable binding geometries. The interaction energy was computed for each of the complexes in their most favourable conformation.

RESULTS AND DISCUSSION

Phase-solubility study

Phase-solubility study is a traditional approach used to measure the stability constants. In addition, it also gives insight into the stoichiometry of the equilibrium (14). In this study, the intrinsic aqueous solubility of vorinostat in pure water was assessed and found to be 191·4 μg/mL ($7{\cdot}24 \times 10^{-1}$ mM), a value consistent with the 'Full Prescription Information' provided by its developer (4). The results were plotted and the phase-solubility diagram of vorinostat complexed in various types of CDs is shown in Fig. 2. Several qualitative observations can be made from the diagram; significant increases in apparent aqueous solubility of vorinostat were obtained when the concentrations of RM-β-CD, HP-β-CD, HP-α-CD and α-CD were increased. Most significantly, RM-β-CD increased the aqueous solubility of vorinostat by approximately 100-folds when vorinostat was complexed in 300 mM RM-β-CD, resulting in a maximal solubility of 70·8 mM (18·7 mg/mL).

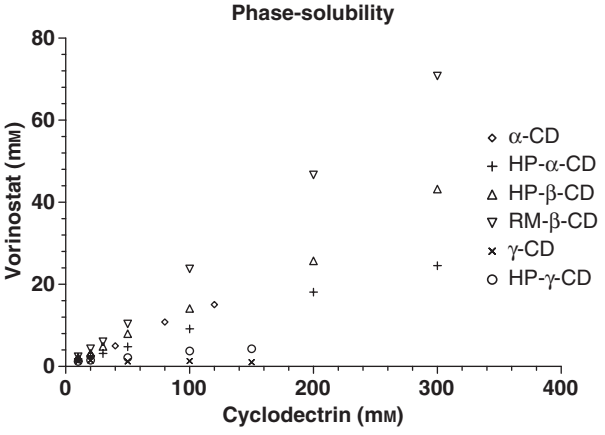

Fig. 2. Phase-solubility study of vorinostat in various CDs.

Similarly, the solubility of vorinostat was increased to 43·2 mM (11·4 mg/mL) in the presence of 300 mM HP-β-CD. In comparison, HP-γ-CD increased the aqueous solubility of vorinostat only 4-fold. At HP-γ-CD concentration higher than 100 mM, the enhancement in solubility of vorinostat was less than twice. The aqueous solubility of vorinostat beyond 150 mM α-CD and 150 mM γ-CD cannot be determined because of limitation in the intrinsic aqueous solubility of these CDs.

The phase-solubility curves of HP-α-CD, α-CD, HP-β-CD, RM-β-CD and HP-γ-CD were linear ($R^2 > 0{\cdot}99$) and displayed a typical A_L type of phase-solubility profile, indicating that the complex formation is first order with respect to the CD and may be first or higher orders with respect to the drug (14). As the slopes of the phase-solubility curves are smaller than 1 in all the CDs, the formation of a 1 : 1 inclusion complex is suggested between vorinostat and the various CDs (14). This justifies the tabulation of the apparent inclusion rate constant ($K_{1:1}$), calculated with the equation established by Higuchi and Connors (12).

The tabulated apparent inclusion rate constant ($K_{1:1}$) values (shown in Table 1) indicated that the complexation affinity between vorinostat and the various CDs are in the following order: RM-β-CD > HP-β-CD > α-CD > HP-α-CD > HP-γ-CD > γ-CD. Hence, in aqueous solutions of vorinostat and CDs, the free drug molecules are in equilibrium with the drug molecules entrapped within the cavities. On increasing the concentration of the CDs, more vorinostat molecules will transfer from the aqueous solution to the hydrophobic cavities of the CD, increasing the apparent solubility of vorinostat.

No covalent bond is generated during the formation of the inclusion complex. Therefore, the binding affinity between CD and vorinostat was not exceptionally strong. In fact, the $K_{1:1}$ values between the CDs and vorinostat were all less than 500/M in this study. From these values, it could be foreseen that upon intravenous administration, HP-β-CD, α-CD or HP-α-CD would not alter the pharmacokinetic profile of vorinostat. The mechanisms of drug release from the CD complexes have also been studied. It has been reported that after intravenous administration, the major driving force for dissociation of weakly to moderately bound drugs in the inclusion complex is simple dilution (15). In most cases, the release of drug molecules from CD complexes is rapid and quantitative (15). Drug/CD complexes are continually forming and dissociating with lifetimes of milliseconds or less (15). Thus, this equilibrium kinetics should not have a significant impact on the elimination kinetics. Moreover, plasma protein binding is also able to induce drug dissociation from the CD-drug complex. Besides, vorinostat binds weakly with CDs but moderately with plasma proteins (approximately 71%) (4). Therefore, HP-β-CD, α-CD or HP-α-CD should work as a parenteral vehicle for vorinostat without changing its pharmacokinetic profile. However, such postulation needs to be confirmed in an actual pharmacokinetic study.

Table 1. Inclusion rate constant between vorinostat and CDs

	α-CD	HP-α-CD	HP-β-CD	RM-β-CD	HP-γ-CD
Cyclodextrin $K_{1:1}$ (M^{-1})	188·7	123·4	218·3	414·8	44·0

Dilution and re-dissolution of vorinostat-HP-β-CD complex

No precipitation was observed in any of the samples with 5-, 10- 50-, 100- and 500-fold dilutions with isotonic phosphate buffer (pH value of 7·4). The freeze-dried product prepared from the solution of vorinostat in 300 mM HP-β-CD was re-dissolved in purified water and then made up to 2 mL. A clear colourless solution of vorinostat was formed and no re-precipitation occurred.

In current clinical practice, vorinostat is only available in the form of 100 mg capsule with a daily oral dose of 400 mg (4). Assuming its oral bioavailability is 43% (16), the intravenous dose of vorinostat would be approximately 200 mg. The aforementioned amount of vorinostat could be administered intravenously in 20 mL of 300 mM HP-β-CD. As the clinical safety for intravenous administration of HP-β-CD has been documented, a parenteral formulation of vorinostat formulated with HP-β-CD is feasible. Such a formulation would provide an alternative dosing route for vorinostat and might improve its therapeutic usage in oncology. Vorinostat is well known for

Inclusion complex	Total energy (kcals/mol)	Steric energy (kcals/mol)	Electrostatic energy (kcals/mol)
Vorinostat-DM-β-CD	−34·5	−17·559	−17·019
Vorinostat -HP-β-CD	−33·2	−17·509	−15·766
Vorinostat -α-CD	−32·4	−17·910	−14·564
Vorinostat -HP-α-CD	−31·4	−17·588	−13·835
Vorinostat - HP-γ-CD	−30·2	−14·950	−15·320
Vorinostat - γ-CD	−29·1	−10·709	−18·444

Table 2. Interaction energies for the complexes formed by vorinostat and various CDs

DM-β-CD, dimethyl-β-cyclodextrin; HP-β-CD, hydroxypropyl-β-cyclodextrin; α-CD, α -cyclodextrin; HP-α-CD, hydroxypropyl- α-cyclodextrin; HP- γ –CD, hydroxypropyl-γ-cyclodextrin; γ-CD, γ –cyclodextrin.

its ability to provide synergistic anti-cancer effects with conventional chemotherapeutic agents in cell culture models (3). Furthermore, the recent oncological trials of vorinostat indicated its values in combination chemotherapy (5). Diarrhoea, nausea and vomiting are common side-effects of cancer chemotherapy. The oral bioavailability of vorinostat can be affected because of these problems and the therapeutic efficacy may be affected. A parenteral formulation of vorinostat could overcome such issues. Oral administration of capsules is also not convenient for the small children or comatose patients. Under such circumstance, an oral liquid of vorinostat formulated with HP-β-CD or RM-β-CD may be useful.

Molecular simulation study

Two different inclusion modes were attempted when the docking was performed. In mode 1, the alkyl chain was introduced into the CD cavity. In mode 2, the benzene ring of the simulated vorinostat molecule was inserted into the CD cavity and allowed to dock freely. After several attempts, the preferred docking mode was found to be mode 1. Fig. 3 shows the hypothetical structures of the complexes between vorinostat and the various CDs. As aforementioned, the alkyl chain of vorinostat is enclosed by the CD and its aromatic ring situated at the rim of the CD cavity. The simulated diagrams suggested that vorinostat and the various CDs undergo a 1 : 1 stoichiometric interaction. Interaction energies of the complexes, including its steric and electrostatic energies, are tabulated and presented in Table 2. The magnitude of the interaction energy indicates the strength of interaction between vorinostat and the respective CD molecule. The strengths of interaction are decreasing in the following order: vorinostat-DM-β-CD > vorinostat-HP-β-CD > vorinostat-α-CD > vorinostat-HP-α-CD > vorinostat-HP-γ-CD > vorinostat-γ-CD.

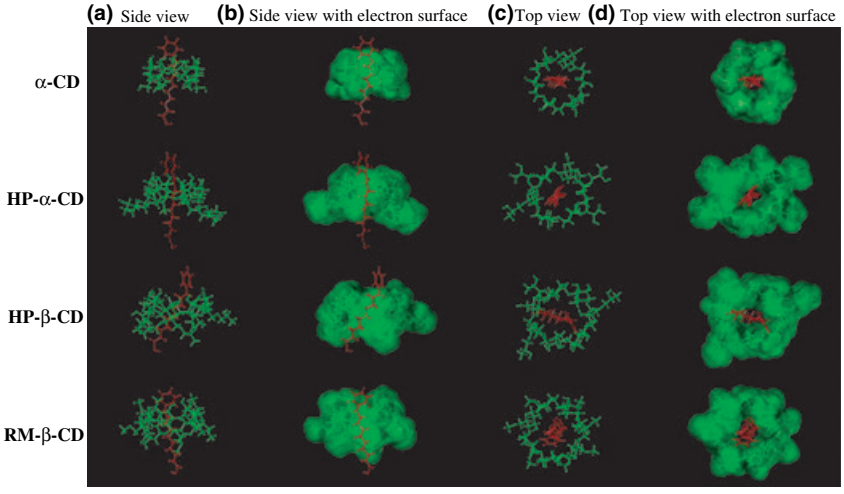

Fig. 3. Hypothetical structure of the vorinostat-CD inclusion complex.

The results obtained from the molecular simulation study were consistent with the findings in the phase-solubility study. This suggests that molecular modelling accurately predicted the differences in binding affinity between vorinostat and the various CDs. Molecular simulation appears to be useful for the selection of appropriate CD as excipient for drug delivery. CDs are generally used to improve the aqueous solubility of guest drug molecules. However, different CDs show different binding affinities for a specific guest molecule. Therefore, it is time-consuming to identify the most appropriate combination of CD and guest drug experimentally. With the rapid development of computational chemistry, the stoichiometric interaction and the interaction energies of the complexes could be predicted *in silico*. Based on such information, the combination with good binding affinity could be selected for further investigation.

CONCLUSION

Cyclodextrins, namely α-CD, HP-α-CD, HP-β-CD, RM-β-CD and HP-γ-CD increased the apparent aqueous solubility of vorinostat in a concentration-dependent manner. RM-β-CD and HP-β-CD enhanced the solubility of vorinostat most. Future studies could focus on HP-β-CD for parenteral delivery of vorinostat or on RM-β-CD as an oral absorption enhancer. Molecular simulation was useful for the selection of an appropriate CD. Further investigations of the biopharmaceutical properties of such formulations are warranted.

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