Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 341 (2007) 238–245
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Note

Biopharmaceutics of 13-*cis*-retinoic acid (isotretinoin) formulated with modified β-cyclodextrins

Hai-Shu Lin, Wendy Wen Yi Leong, Jie An Yang, Pei Lee, Sui Yung Chan, Paul C. Ho *

Department of Pharmacy, Faculty of Science, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260, Singapore

Received 25 January 2007; received in revised form 19 March 2007; accepted 31 March 2007
Available online 5 April 2007

Abstract

13-*cis*-Retinoic acid (13-*cis*-RA), also known as isotretinoin, is commonly used in the management of severe acne. Its clinical efficacy in oncology has also been documented. As a vitamin A derivative, it is not soluble in water. This solubility barrier not only affects its oral absorption but also makes parenteral delivery difficult. Recently, water-soluble formulations of 13-*cis*-RA have been attempted with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) and randomly methylated-β-cyclodextrin (RM-β-CD). In this study, the pharmacokinetic profiles of these two formulations were assessed in Sprague–Dawley rats after single intravenous or oral administration. We found that 13-*cis*-RA was eliminated from the body through a dose-independent process after intravenous injection of either sodium salt or the HP-β-CD formulation within the tested dosage range (2.0–7.5 mg/kg). Furthermore, HP-β-CD did not alter the kinetic profile of 13-*cis*-RA after intravenous administration in comparison with 13-*cis*-RA sodium salt. We also found that RM-β-CD dramatically enhanced the oral absorption of 13-*cis*-RA. At 10.0 mg/kg, the bioavailability of 13-*cis*-RA formulated with RM-β-CD was about three-fold higher than that of the control (13-*cis*-RA suspended in 0.5% carboxymethylcellulose (CMC)). Similarly, the oral absorption of 13-*cis*-RA was not saturated within our tested range (2.5–10.0 mg/kg) and the bioavailability remained unchanged. These results demonstrated that HP-β-CD and RM-β-CD were suitable excipients for the delivery of 13-*cis*-RA.

Keywords: 13-*cis*-Retinoic acid; Isotretinoin; Pharmacokinetics; Modified β-cyclodextrin

1. Introduction

13-*cis*-Retinoic acid (13-*cis*-RA), also known as isotretinoin, is an endogenous vitamin A derivative (Tang and Russell, 1990). It is commonly used in dermatology for the management of cystic acne, recalcitrant nodular acne, as well as several other skin disorders (Ward et al., 1984; Gollnick et al., 1990). Besides its dermatological indications, the clinical efficacy of 13-*cis*-RA in oncology has also been documented. It could be used for the treatment of oral leukoplakia (pre-malignant lesion) (Hong et al., 1986); as a chemoprophylaxis, 13-*cis*-RA effectively prevented the second primary tumors in patients who have been treated for squamous cell carcinoma and the skin cancers in patients with xeroderma pigmentosum (Kraemer et al., 1988; Hong et al., 1990). In other studies, the therapeutic efficacy of 13-*cis*-RA was demonstrated in juvenile chronic myelogenous leukemia (Castleberry et al., 1994), high-risk neuroblastoma (Matthay et al., 1999), advanced skin squamous cell carcinoma (Aass et al., 2005), and progressive metastatic renal cell carcinoma (Shin et al., 2002).

Being a lipophilic retinoid, the aqueous solubility of 13-*cis*-RA is almost nil, making it difficult for parenteral delivery. For systemic administration, 13-*cis*-RA is usually given through repeated oral dosing. However, the estimated bioavailability was only approximately 25% (Gollnick et al., 1990). Partially due to first-pass effects, such limited oral bioavailability could be attributed to its poor solubility. As 13-*cis*-RA is almost insoluble in aqueous solution, its oral absorption could be affected by the pH value of gastric intestinal fluid, the fatty acid composition in the diet, and the excretion of bile. Therefore, the pharmacokinetic profile of 13-*cis*-RA after oral drug administration is highly variable (Ward et al., 1984).

Cyclodextrins (CDs), cyclic oligosaccharides derived from starch, are well known for their abilities to form inclusion complexes with various guest molecules (Davis and Brewster, 2004). Inclusion of hydrophobic drugs into cyclodex-

* Corresponding author. Tel.: +65 6516 2651; fax: +65 6779 1554.
E-mail address: phahocl@nus.edu.sg (P.C. Ho).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.03.050

trins usually leads to an increase in aqueous solubility, improvement in oral bioavailability, and enhanced chemical stability (Davis and Brewster, 2004). It has been well documented that cyclodextrins, especially β-cyclodextrin derivatives, were able to form inclusion complexes with retinoids and such cyclodextrin-based formulations displayed beneficial effects. For example, 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) increased the aqueous solubility, photo-stability as well as the oral bioavailability of all-*trans*-retinoic acid (all-*trans*-RA, tretinoin) (Lin et al., 2000a,b); similarly, both HP-β-CD and randomly methylated-β-cyclodextrin (RM-β-CD) improved the aqueous solubility and photo-stability of acitretin while RM-β-CD dramatically enhanced the oral absorption of acitretin (Liu et al., 2003, 2004). As HP-β-CD is an injectable excipient (Davis and Brewster, 2004), parenteral delivery of all-*trans*-RA, all-*trans*-retinoyl-β-D-glucuronide, and acitretin becomes possible (Lin et al., 2000a, 2001; Liu et al., 2004). The physicochemical properties of 13-*cis*-RA-cyclodextrin complexes have also been evaluated (Yap et al., 2005). Similar to its effects on the all-*trans* isomer, HP-β-CD enhanced the aqueous solubility and photo-stability of 13-*cis*-RA (Yap et al., 2005). However, the biopharmaceutical properties of this cyclodextrin complex have yet to be assessed. Furthermore, the delivery of 13-*cis*-RA with the aid of RM-β-CD has never been attempted. When compared with HP-β-CD, methylated cyclodextrins, such as RM-β-CD, usually have superior effects on the aqueous solubility and the oral bioavailability of the complexed drug. Therefore, we hypothesized that HP-β-CD could be used as a vehicle for the parenteral delivery of 13-*cis*-RA, while RM-β-CD could enhance the systemic exposure of 13-*cis*-RA after oral administration. To prove this hypothesis, the pharmacokinetics of 13-*cis*-RA formulated with HP-β-CD and RM-β-CD, respectively, was studied in Sprague–Dawley rats. Our results demonstrated that HP-β-CD and RM-β-CD were suitable excipients to improve the delivery of 13-*cis*-RA.

2. Materials and methods

2.1. Special precaution

All laboratory procedures involving the manipulations of 13-*cis*-RA were executed in a dimly lit environment to prevent its photo-isomerization.

2.2. Materials

13-*cis*-Retinoic acid was purchased from Toronto Research Chemicals (North York, Ontario, Canada). 2-Hydroxypropyl-β-cyclodextrin (degree of substitution: about 0.6) and randomly methylated-β-cyclodextrin (degree of substitution: about 1.8) was kindly donated from Roquette (Lestrem, France) and Wacker (Burghausen, Germany), respectively. HPLC grade methanol and acetonitrile were obtained from Tedia (Fairfield, OH 45014, USA). Ammonium acetate and the sodium salt of carboxymethylcellulose (CMC) were purchased from Merck (Darmstadt, Germany) and Sigma (St. Louis, MO 63178, USA), respectively. Purified water (18.2 MΩ cm at 25 °C) was generated from a Millipore Direct-Q® ultra-pure water system (Billerica, MA 01821, USA) and used throughout the study.

2.3. High-performance liquid chromatographic (HPLC) assay

A Shimadzu (Kyoto, Japan) 2010A liquid chromatography was used for the analysis. The separation was done on a reversed-phase HPLC column (ODS Hypersil, 5 μm, 250 mm × 4 mm, Agilent, Palo Alto, CA 94306, USA), which was protected with a mechanical filter (Rheodyne, Cotati, CA 94928, USA) and a guard column (Agilent). The assay was performed at 60 °C through isocratic delivery of the mobile phase, consisting of acetonitrile–methanol–1% ammonium acetate (20:55:25, v/v). The flow rate was set at 1.5 ml/min. 13-*cis*-RA was quantitated by measuring UV absorbance at 350 nm. For sample preparation, 300 μl acetonitrile was added to 100 μl plasma. After vigorous vortex, the sample was centrifuged at 5000 × *g* for 5 min. The supernatant was then placed into an amber autosampler vial and 50 μl of the supernatant was injected into the HPLC. The limit of quantitation (LOQ) of the assay was 10 ng/ml. 13-*cis*-RA was calibrated through an external standard method. The calibration curve, obtained by spiking 13-*cis*-RA into pooled rat plasma, was linear ($R^2 > 0.999$) within the range of 10–1000 ng/ml. The intra-day and inter-day variation were all less than 7% and the recovery rate of 13-*cis*-RA in plasma was >96%. For the pharmacokinetic study, plasma samples with high 13-*cis*-RA concentration were properly diluted to within our calibration range (10–1000 ng/ml) before HPLC analysis. Similarly, those concentrated samples from the formulation preparation were diluted with methanol and only 5 μl diluted samples were injected into the HPLC. The calibration range for the formulation samples was 1–50 μg/ml. This HPLC assay method was a slight modification of the previously described methods (Lin et al., 2000a, 2001; Liu et al., 2004; Yap et al., 2005).

2.4. Preparation of dosing formulations

The sodium salt of 13-*cis*-RA for intravenous injection was prepared by dissolving 15 mg of 13-*cis*-RA in 10 ml of 0.9% NaCl–0.3% NaOH (w/v) solution according to the previously described procedure (Guchelaar et al., 1992). This solution was clear; therefore 13-*cis*-RA was completely dissolved. Such dosing solution was freshly prepared daily. The 13-*cis*-RA–HP-β-CD inclusion complex solution for intravenous injection was prepared as follow: 100 mg of 13-*cis*-RA was suspended in 10 ml of 0.067 M (pH 7.4) phosphate buffer containing 0.3 M HP-β-CD, the suspension was sonicated for 1 h and then shaken on a horizontal rotary shaker for 8 days at a speed of 300 rpm at ambient temperature. Finally, the suspension was filtered through a 0.22 μm syringe driven filter (Millipore, Billerica, MA 01821, USA). A sample of the clear solution was diluted 100 times with methanol and injected into HPLC to quantitate its 13-*cis*-RA concentration. The method for the preparation of 13-*cis*-RA–HP-β-CD inclusion complex solution was modified

from a previous study (Yap et al., 2005). The 13-*cis*-RA–RM-β-CD inclusion complex solution for oral administration was prepared in the similar way as described above. The 13-*cis*-RA suspension for oral dosing was prepared by suspending 15 mg13-*cis*-RA into 5 ml 0.5% CMC (pH 7.4). The suspension was shaken vigorously before oral gavage.

2.5. Animals

Adult male Sprague–Dawley rats (250–300 g) were obtained from the Laboratory Animal Center of the National University of Singapore. The rats were maintained on a 12-h light/dark cycle. No restriction to food and water was applied to the rats that received intravenous administration of 13-*cis*-RA. Rats that received oral administration were fasted overnight before the study and no food was provided up to 6 h after oral gavage. On the day before the pharmacokinetic study, a polyethylene tube (i.d. 0.58 mm, o.d. 0.965 mm, Becton Dickinson, Sparks, MD 21152, USA) was placed into the jugular vein under anesthesia. This cannula was used for intravenous drug administration as well as blood sample collection. The rats were randomly divided into 10 groups; six groups (each group with four rats) received intravenous administration of 13-*cis*-RA and four groups (each group with six rats) received oral dosing through gavage. Rats in Groups 1, 3, and 5 received a single bolus intravenous injection of 13-*cis*-RA sodium salt solution (1.5 mg/ml) at the dose of 2.0, 4.0, and 7.5 mg/kg, respectively. Blood samples were collected at 10 min, 20 min, 40 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, and 10 h after intravenous injection. Rats in Group 2, 4, and 6 received the same doses as Group 1, 3, and 5, respectively, as 13-*cis*-RA–HP-β-CD solution at the same concentration of 1.5 mg/ml. Both Groups 7 and 8 received a single oral administration of 13-*cis*-RA at the dose of 10.0 mg/kg. For, in Group 7, 13-*cis*-RA was suspended in 0.5% CMC (3 mg/ml, pH 7.4); while for Group 8, 13-*cis*-RA–RM-β-CD (3 mg/ml, pH 7.4) was administered. Similarly, 13-*cis*-RA–RM-β-CD was given to Groups 9 and 10 at the dose of 5.0 and 2.5 mg/kg, respectively. For group 7, blood samples were collected at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, and 10 h; while for Groups 8, 9, and 10, blood samples were collected at 15 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, and 10 h. After each intravenous injection or blood sampling, 0.3 ml heparin–saline (5 μ/ml) was used to flush the cannula. The blood samples were centrifuged at 5000 × *g* for 5 min, and the plasma was collected and stored at −80 °C until HPLC analysis. This study was performed according to the guidelines for the humane use of animals in scientific research. This animal experimental protocol was modified from several previous studies (Lin et al., 2000a, 2001; Liu et al., 2004); and approved by the Institutional Animal Care and Use Committee of the National University of Singapore.

2.6. Pharmacokinetic analysis

All compartmental and non-compartmental pharmacokinetic parameters were obtained with the software WinNonlin standard Version 1.0 (Scientific Consulting Inc., Apex, NC 27502, USA). The area under the plasma concentration–time curve ($AUC_{0\to t}$) in rats that received oral administration (Groups 7, 8, 9, and 10) was calculated by the trapezoidal rule with the time point from 0 to the last detectable time point, whereas the AUC in rats that received intravenous dosing (Groups 1–6) was calculated through the same rule except the logarithmic scale was taken. Similarly, clearance (Cl) in Groups 1–6 was also calculated through non-compartmental method as: $Cl = dose/AUC_{0\to t}$. The absolute bioavailability (F) values of 13-*cis*-RA in Groups 7–10 was calculated as

$$F\,(\%) = \frac{AUC_{0\to t}(\text{Groups 7, 8, 9, or 10})/\text{dose (Groups 7, 8, 9, or 10)}}{AUC_{0\to t}(\text{Group 1})/2\ \text{mg kg}^{-1}} \times 100\%.$$

Because a distribution phase followed by a prolonged terminal elimination phase was observed after intravenous dosing in all of the tested rats, the plasma 13-*cis*-RA concentration–time data was fitted into the classical two-compartment first-order open model ($C = Ae^{-\alpha t} + Be^{-\beta t}$) using nonlinear least squares curve fitting with a weighting factor of $1/Y^2$ as described previously (Lin et al., 2001; Liu et al., 2004).

2.7. Statistics

Statistical analysis was conducted with the software GraphPad Prism Version 2.00 (San Diego, CA 92130, USA). All experimental data were expressed as mean ± standard deviation (S.D.) except time to maximal concentration (T_{max}) after oral administration, because it was a non-continuous data due to the pre-decided sampling schedule. Two-tail independent sample *t*-test was used to compare the pharmacokinetic parameters between the two different formulations at the same dose (13-*cis*-RA sodium salt *versus* 13-*cis*-RA–HP-β-CD, 13-*cis*-RA suspended in 0.5% CMC *versus* 13-*cis*-RA–RM-β-CD). One-way ANOVA with the *post hoc* Tukey test was used to compare the pharmacokinetic parameters among the three doses with the same formulations (13-*cis*-RA sodium salt, 13-*cis*-RA–HP-β-CD, or 13-*cis*-RA–RM-β-CD). Kruskal–Wallis test with the *post hoc* Dunn test and two-tail Mann–Whitney test were used to compare the T_{max} among Groups 8, 9, and 10 and between Groups 7 and 8, respectively. A p value less than 0.05 was adopted to indicate statistically significant difference.

3. Results

3.1. Pharmacokinetics after intravenous administration

The plasma pharmacokinetic profiles of 13-*cis*-RA after intravenous administration are shown in Fig. 1. After intravenous injection, 13-*cis*-RA appeared to be eliminated from the body through a bi-exponential process. Therefore, the plasma 13-*cis*-RA concentration *versus* time data of individual rat was fitted into the classical two-compartment first-order elimination model. However, in agreement with the previous published literatures (Gollnick et al., 1990), we noted that 13-*cis*-RA had a fairly long terminal elimination half-life. Furthermore, secondary peaks in the plasma 13-*cis*-RA concentration *versus* time curve was observed in half of the animals at about 6–10 h after intravenous drug administration. Thus, it is difficult to make

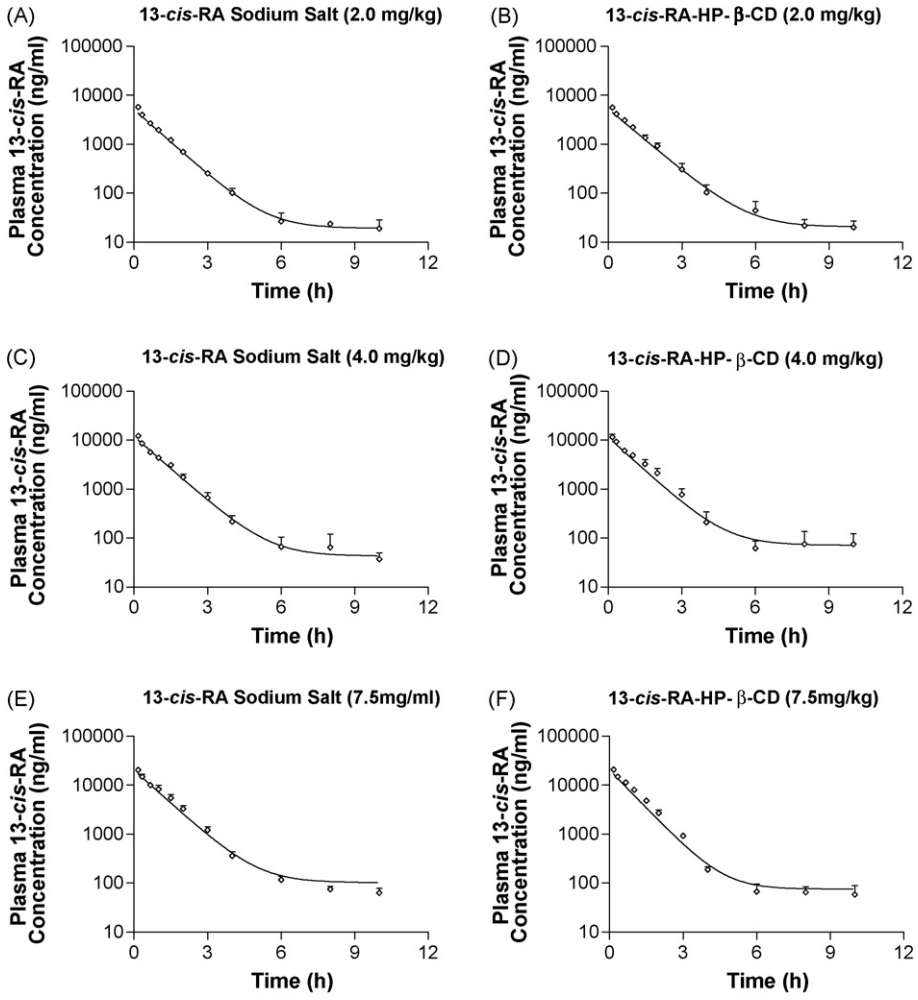

Fig. 1. Pharmacokinetics of 13-*cis*-RA after a single intravenous administration. The line represents the predicted values. Symbols represent the mean observed values ($n = 4$); error bar represent the S.D.

accurate estimation of the model-dependent pharmacokinetic parameters, including the elimination rate constant (K_e), the elimination half-life ($t_{1/2}$), and distribution rate constant from central/peripheral compartment to peripheral/central compartment ($K_{1:2}/K_{2:1}$), in about half of the animals. So, the only model-dependent pharmacokinetic parameter obtained from the modeling was the volume of distribution of the central compartment (V_c), which had small calculation error in all rats (CV all less than 17%). The area under the plasma concentration–time curve ($AUC_{0\rightarrow t}$) and clearance (Cl) were obtained through non-compartmental methods. All these pharmacokinetic parameters are listed in Table 1.

After single intravenous administration of 13-*cis*-RA sodium salt, the V_c value in Groups 1, 3, and 5 were very similar (Group 1, 3, and 5: 322 ± 17, 304 ± 11, and 328 ± 40 ml/kg, respectively). The dose appeared to have no significant impact on the distribution of 13-*cis*-RA (one-way ANOVA, $p > 0.05$). The values of AUC of 13-*cis*-RA in Groups 1, 3, and 5 (dose: 2.0, 4.0, 7.5 mg/kg) were proportional to the dose and the Cl remained unchanged (one-way ANOVA, $p > 0.05$). Similar

Table 1
Pharmacokinetic parameters of 13-*cis*-RA after intravenous administration

	Parameters					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Formulation	Sodium salt	HP-β-CD	Sodium salt	HP-β-CD	Sodium salt	HP-β-CD
Dose (mg kg^{-1})	2.0	2.0	4.0	4.0	7.5	7.5
V_c (ml kg^{-1})	322 ± 17	308 ± 43	304 ± 11	273 ± 26	328 ± 40	283 ± 22
AUC (ng h ml^{-1})	5922 ± 271	6464 ± 362	13280 ± 1430	13949 ± 1682	23226 ± 1954	22319 ± 1097
Cl (ml h kg^{-1})	338 ± 15	310 ± 18	304 ± 33	290 ± 36	322 ± 26	334 ± 16

$n = 4$, data is presented as mean ± S.D.

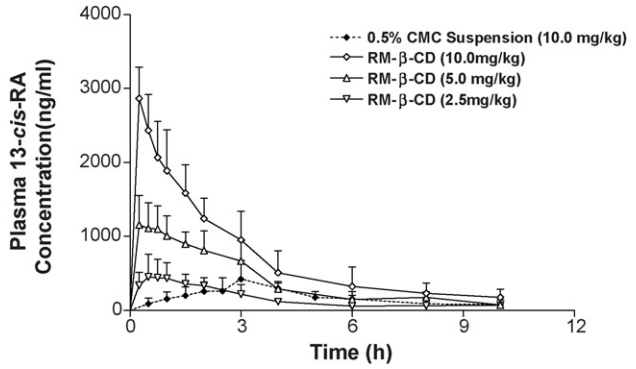

Fig. 2. Pharmacokinetics of 13-*cis*-RA after a single oral administration. Symbols represent the mean values of the group ($n = 6$); error bar represent the S.D.

results were also observed in the rats that received a single intravenous administration of 13-*cis*-RA–HP-β-CD solution. The values of AUC of 13-*cis*-RA RA in Groups 2, 4, and 6 were proportional to the dose, while the values of V_c and Cl remained unchanged (one-way ANOVA, $p > 0.05$). Therefore, after a single intravenous administration, 13-*cis*-RA was eliminated through a dose-independent process within the dosing range of 2.0–7.5 mg/kg.

Although HP-β-CD dramatically enhanced the aqueous solubility of 13-*cis*-RA, it did not alter the pharmacokinetic profile after intravenous administration in comparison to 13-*cis*-RA sodium salt. The plasma 13-*cis*-RA concentration *versus* time curves (Fig. 1) of Groups 2, 4, and 6 are very similar to those of Group 1, 3, and 5, respectively, suggesting an unchanged pharmacokinetic profile. Furthermore, the values of AUC, the V_c and the Cl between 13-*cis*-RA sodium salt and 13-*cis*-RA–HP-β-CD were all similar (independent sample *t*-test: $p > 0.05$). Therefore, HP-β-CD did not have a significant impact on the pharmacokinetics of 13-*cis*-RA after intravenous administration within the dosing range of 2.0–7.5 mg/kg.

3.2. Pharmacokinetics after oral administration

The plasma kinetic profiles of 13-*cis*-RA after oral dosing are shown in Fig. 2. The pharmacokinetic parameters were calculated with non-compartmental method and are listed in Table 2. A delayed absorption was observed after a single oral gavage of 13-*cis*-RA suspended in 0.5% CMC (pH 7.4) at the dose of 10.0 mg/kg (Group 7). The plasma 13-*cis*-RA concentration increased slowly and the time to maximal concentration (T_{max}) was either 3 or 4 h (3 h: four rats, 4 h: two rats). The maximal concentration (C_{max}) was only 441 ± 226 ng/ml. The absolute bioavailability (F) was as low as $5.9 \pm 2.2\%$. In summary, the 13-*cis*-RA suspension was slowly absorbed with very low bioavailability. In contrast to the CMC suspension, 13-*cis*-RA fully solublized with RM-β-CD had an ultra-high absorption rate. In Group 8 (10.0 mg/kg), 15 min after oral gavage (the first sample time point), maximum concentration was observed in five out of the total six rats, making the compartmentally based pharmacokinetic modeling difficult. Therefore, the absorption rate constant (K_a) was not estimated. Although the T_{max} value of the 13-*cis*-RA–RM-β-CD was much shorter than that of the suspension (two-tail Mann–Whitney test, $p < 0.001$), its C_{max} was about five-fold higher than that of the CMC suspension (2911 ± 386 *versus* 441 ± 226 ng/ml, two-tail independent sample *t*-test: $p < 0.0001$). Most importantly, the 13-*cis*-RA–RM-β-CD led to a three-fold enhancement in oral bioavailability when compared to the CMC suspension ($24.6 \pm 7.3\%$ *versus* $5.9 \pm 2.2\%$, two-tail independent sample *t*-test: $p < 0.0001$). In summary, RM-β-CD increased both the speed and the extent of the oral absorption of 13-*cis*-RA.

The absorption rate at 5.0 mg/kg (Group 9) was observed to be slightly slower than that at 10.0 mg/kg (Group 8) and its C_{max} was observed up to 1 h after dosing. However, such difference was not statistically significant (two-tail Mann–Whitney test: $p = 0.1198$). As the dose dropped further to 2.5 mg/kg in Group 10, decreased absorption speed was observed. Although no statistically significant difference in T_{max} between Groups 10 and 9 was observed, the T_{max} of Group 10 was significantly longer than that of Group 8 (Kruskal–Wallis test with the *post hoc* Dunn test: $p < 0.05$). Interestingly, the extent of absorption of 13-*cis*-RA solublized with RM-β-CD appeared to be dose-independent and no significant difference in bioavailability was observed among Groups 8, 9, and 10 (one-way ANOVA, $p > 0.05$). Therefore, the oral absorption of 13-*cis*-RA was not saturated within the dose 2.5–10.0 mg/kg.

Table 2
Pharmacokinetic parameters of 13-*cis*-RA after oral administration

	Parameters			
	Group 7	Group 8	Group 9	Group 10
Formulation	CMC Suspension	RM-β-CD	RM-β-CD	RM-β-CD
Dose (mg kg^{-1})	10.0	10.0	5.0	2.5
T_{max} (h)	3 or 4	0.25 or 0.5**	0.25–1	0.25–3*
C_{max} (ng ml^{-1})	441 ± 226	2911 ± 386***	1211 ± 365	561 ± 260
AUC (ng h ml^{-1})	1757 ± 638	7281 ± 2156***	4113 ± 871	1616 ± 274
F (%)	5.9 ± 2.2	24.6 ± 7.3***	27.8 ± 5.9	21.8 ± 3.7

$n = 6$, data is presented as mean ± S.D. except T_{max}.
* $p < 0.05$ between this group and Group 8.
** $p < 0.001$ between this group and Group 7.
*** $p < 0.0001$ between this group and Group 7.

4. Discussion

Due to the lack of an injectable formulation, the pharmacokinetics of 13-*cis*-RA after intravenous administration has not been assessed in humans. Thus, the absolute bioavailability in humans is still unknown, despite the fact that it has been used clinically for more than 25 years. In this study, we found that HP-β-CD was a suitable carrier for 13-*cis*-RA. As the safety of HP-β-CD for intravenous application has been documented (Davis and Brewster, 2004), parenteral delivery of 13-*cis*-RA in human is possible. An injectable formulation of 13-*cis*-RA provides an alternative route to deliver 13-*cis*-RA. This is potentially beneficial to the cancer patients as their digestive system may be impaired due to the illness or the treatments.

The kinetic profile of 13-*cis*-RA after intravenous administration has been studied in various pre-clinical models including mouse (Wang et al., 1980), rat (Guchelaar et al., 1992), guinea pig (Chien et al., 1992), rabbit (Huselton et al., 1996), dog (Cotler et al., 1983), and monkey (Sandberg et al., 1994). Generally, after intravenous dosing, a distribution phase followed by a terminal elimination phase is observed in the drug plasma/serum *versus* time curve. Our findings were in agreement to the findings of the previous studies. Similarly, the Cl of 13-*cis*-RA observed in Sprague–Dawley rat was within the same scale when compared with that of the rabbit, dog, and monkey (Patel et al., 1982; Cotler et al., 1983; Sandberg et al., 1994; Huselton et al., 1996); but it was about three-fold lower than that observed in guinea pig (Chien et al., 1992). In our study, we found that 13-*cis*-RA was eliminated from the body through a dose-independent process (AUCs were proportional to the doses) and clearance remained unchanged. A similar result was also obtained in monkeys (Sandberg et al., 1994). We observed secondary peaks in the plasma 13-*cis*-RA concentration *versus* time curve in half of the animals at about 6–10 h after intravenous drug administration. Such secondary peaks were probably due to enterohepatic circulation, as biliary clearance 13-*cis*-RA has been reported previously (Cotler et al., 1983; Colburn et al., 1985; Meloche and Besner, 1986). In fact, such double peak phenomenon has also been observed before in rats, guinea pigs, and rabbits (Liu et al., 1990; Chien et al., 1992; Huselton et al., 1996).

In our study, we found that HP-β-CD does not significantly alter the pharmacokinetic profile of 13-*cis*-RA after intravenous administration when compared to 13-*cis*-RA sodium salt (Fig. 1 and Table 1). This phenomenon can be explained by the moderate binding affinity between 13-*cis*-RA and HP-β-CD and high binding affinity between 13-*cis*-RA and albumin. In a previous discussion on the mechanisms of drug release from cyclodextrin complexes, Stella et al. suggested that after parenteral administration, the major driving force for dissociation of weakly to moderately bound drugs in the inclusion complex is simply dilution (Stella et al., 1999). It has also been noted that drug release from cyclodextrin complexes is rapid and quantitative in most cases; in aqueous solution, drug/cyclodextrin complexes are continually forming and dissociating with lifetimes in the range of milliseconds or less (Stella et al., 1999). Therefore, such a short period of time should not result in differences in pharmacokinetics. Furthermore, protein binding may also facilitate drug dissociation from the cyclodextrin–drug complex (Frijlink et al., 1991; Stella et al., 1999). 13-*cis*-RA–HP-β-CD has a moderate inclusion stability constant (about $1.4 \times 10^4\ M^{-1}$), which was determined by phase-solubility method (Yap et al., 2005). Also, its binding with albumin is as high as 99.9% (Brazzell and Colburn, 1982). Thus, upon intravenous administration, 13-*cis*-RA is expected to be released rapidly from the HP-β-CD inclusion complex and bind well to the plasma proteins. Similar results were also observed in several parenteral formulations developed with HP-β-CD or sulfobutylether-β-cyclodextrin (SBE_7-β-CD), where the binding affinity (expressed as inclusion stability constant) between the drug and the cyclodextrin was weak or moderate (Frijlink et al., 1991; Piel et al., 1999; Lin et al., 2000a). Such formulations include all-*trans*-RA–HP-β-CD ($2.7 \times 10^2\ M^{-1}$), miconazole–HP-β-CD ($1.1 \times 10^2\ M^{-1}$), miconazole–SBE_7-β-CD ($1.7 \times 10^2\ M^{-1}$), naproxen–HP-β-CD ($2.2 \times 10^3\ M^{-1}$), and flurbiprofen–HP-β-CD ($1.3 \times 10^4\ M^{-1}$) (Frijlink et al., 1991; Piel et al., 1999; Lin et al., 2000a). However, for the molecule that has high binding affinity with cyclodextrin, the impact of cyclodextrin on its intravenous pharmacokinetics is not neglectable. For example, after intravenous infusion of a synthetic ozonide antimalarial complexed with 0.1 M SBE_7-β-CD (inclusion stability constant: $2.3 \times 10^6\ M^{-1}$), there was an 8.5-fold decrease in the steady-state blood volume of distribution, a 6.6-fold decrease in the mean residence time, and a greater than 200-fold increase in renal clearance in comparison to a cyclodextrin-free isotonic buffered glucose formulation (Charman et al., 2006).

It has been noted that the oral absorption of most retinoids were increased with food and differing dietary regimens may contribute to the wide intra- and inter-patient variations in peak plasma 13-*cis*-RA concentration curve (Ward et al., 1984; Gollnick et al., 1990). It is very possible that the oral absorption of 13-*cis*-RA depends on the pH, fatty acid composition and the inherent bile salt solubilization capacity in the intestine. To avoid the influence of these factors, the experimental animals in Groups 7–10 were kept fasted from the pre-experiment evening to 6 h after dosing.

In our study, we demonstrated that 13-*cis*-RA–RM-β-CD dramatically enhanced the speed and the extent of the oral absorption of 13-*cis*-RA. The mechanisms of oral absorption of small molecules have been well studied. The oral absorption of a drug is influenced by many different biological and physicochemical factors (Lin and Lu, 1997). It has been well demonstrated that the two most important physicochemical factors that affect both the extent and the rate of absorption are lipophilicity and solubility (Lin and Lu, 1997). Since the membrane of the gastrointestinal epithelial cells is composed of tightly packed phospholipids interspersed with proteins, the transcellular passage of drugs depends on their permeability characteristics to penetrate the lipid bi-layer of the epithelial cell membrane, which in turn depends on the lipophilicity of the drugs (Lin and Lu, 1997). The lipophilicity required for good intestinal absorption is not a problem in 13-*cis*-RA, because it is a highly lipophilic fatty acid with a log D value of 4 (determined in *n*-octanol/10 mM phosphate buffer (pH 7.4)) (Chien

et al., 1992). Furthermore, its other key physiochemical properties, including molecular weight (300.4 g/mol), hydrogen donors and receptors (1 and 2, respectively), polar surface area (37.3 Å) (predicted by SciFinder Scholar. Version 2006), rotatable bonds (5) do not prompt issues in bio-membrane permeability. However, the aqueous solubility of 13-*cis*-RA is almost nil, posing a significant problem and affecting its absorption in gastric intestinal tract. In Group 7 (13-*cis*-RA suspended in 0.5% CMC, pH 7.4), an obvious delayed absorption of 13-*cis*-RA was observed, probably a reflection of the slow dissolution of 13-*cis*-RA in the gastric intestinal tract. However, when 13-*cis*-RA was fully solubilized by RM-β-CD (Group 8), the plasma 13-*cis*-RA concentration increased very rapidly and reached peak concentration at 15 or 30 min after dosing. Furthermore, the C_{max} and F were five- and three-fold, respectively, higher than those in Group 7. This can be explained by the difference in solubility of 13-*cis*-RA in the two groups. A suspension of 13-*cis*-RA in 0.5% CMC is insoluble in gastric acid and only slightly soluble in the weakly alkaline intestinal fluid over a long period of time. This may have resulted in the decrease in rate and extent of oral absorption. When 13-*cis*-RA was formulated as an RM-β-CD complex for oral administration, its solubility in the gastric intestinal fluid solution was greatly improved, resulting in an immediate, rapid and more thorough absorption.

We observed a decrease in absorption rate when the dose was decreased from 10.0 to 2.5 mg/kg. This subsequently led to an increase in the T_{max}. Such a phenomenon can also be explained by the solubility issue. We administered 13-*cis*-RA–RM-β-CD solution orally at the concentration of 3 mg/ml to the rats in Groups 8, 9, and 10. However, this was not a saturated solution. According to our phase solubility study (our unpublished data), 0.3 M RM-β-CD (pH 7.4) can form a 13-*cis*-RA solution of 4.5 mg/ml. Therefore, the 13-*cis*-RA–RM-β-CD (3 mg/ml) still had one-third unsaturated buffer capacity. Upon oral gavage, the 13-*cis*-RA–RM-β-CD would mix with the stomach fluid, which is acidic. When a large amount of 13-*cis*-RA–RM-β-CD is given, e.g. at the dose of 10.0 mg/kg, 13-*cis*-RA might not precipitate out in the stomach acid due to the unsaturated buffer capacity. After gastric emptying, the solution entered the small intestine, resulting in an immediate rapid absorption. On the other hand, when less 13-*cis*-RA–RM-β-CD was given, e.g. at the dose of 2.5 mg/kg, part of the dosed 13-*cis*-RA may precipitate out as the buffer capacity was insufficient to maintain the solubility of 13-*cis*-RA. After the 13-*cis*-RA precipitate goes into the small intestine, where it is slightly alkaline, it will re-dissolve and be absorbed. Hence, a delay in the absorption is observed. This might be the reason behind the prolonged absorption period in Groups 9 and 10. Fortunately, the delayed absorption did not have a significant impact on the bioavailability, indicating that the oral absorption of 13-*cis*-RA was not saturated within our tested range of 2.5–10.0 mg/kg. Similar results have also been observed in humans. Colburn and Gibson found that the C_{max} and AUC were essentially dose related in the range of 80–240 mg (Colburn and Gibson, 1985). However, saturation was observed at higher dose of 320 mg (Colburn and Gibson, 1985).

In this study, the biopharmaceutics of 13-*cis*-RA formulated with modified β-cyclodextrins was assessed in Sprague–Dawley rats after intravenous or oral administrations. We found that HP-β-CD did not alter the kinetic profile of 13-*cis*-RA upon intravenous administration. As HP-β-CD is an injectable excipient, it could work as a vehicle for the parenteral delivery of 13-*cis*-RA, which has never been attempted in humans. Since RM-β-CD dramatically improved the oral bioavailability of 13-*cis*-RA, it appeared to be a proper excipient for the oral delivery of 13-*cis*-RA. However, these postulations warrant further investigations.

Acknowledgement

We are grateful to the financial support from Academic Research Fund awarded from the National University of Singapore.

References

Aass, N., De Mulder, P.H., Mickisch, G.H., Mulders, P., van Oosterom, A.T., van Poppel, H., Fossa, S.D., de Prijck, L., Sylvester, R.J., 2005. Randomized phase II/III trial of interferon Alfa-2a with and without 13-*cis*-retinoic acid in patients with progressive metastatic renal cell carcinoma: the European Organisation for Research and Treatment of Cancer Genito-Urinary Tract Cancer Group (EORTC 30951). J. Clin. Oncol. 23, 4172–4178.

Brazzell, R.K., Colburn, W.A., 1982. Pharmacokinetics of the retinoids isotretinoin and etretinate. A comparative review. J. Am. Acad. Dermatol. 6, 643–651.

Castleberry, R.P., Emanuel, P.D., Zuckerman, K.S., Cohn, S., Strauss, L., Byrd, R.L., Homans, A., Chaffee, S., Nitschke, R., Gualtieri, R.J., 1994. A pilot study of isotretinoin in the treatment of juvenile chronic myelogenous leukemia. N. Engl. J. Med. 331, 1680–1684.

Charman, S.A., Perry, C.S., Chiu, F.C., McIntosh, K.A., Prankerd, R.J., Charman, W.N., 2006. Alteration of the intravenous pharmacokinetics of a synthetic ozonide antimalarial in the presence of a modified cyclodextrin. J. Pharm. Sci. 95, 256–267.

Chien, D.S., Sandri, R.B., Tang-Liu, D.S., 1992. Systemic pharmacokinetics of acitretin, etretinate, isotretinoin, and acetylenic retinoids in guinea pigs and obese rats. Drug Metab. Dispos. 20, 211–217.

Colburn, W.A., Gibson, D.M., 1985. Isotretinoin kinetics after 80 to 320 mg oral doses. Clin. Pharmacol. Ther. 37, 411–414.

Colburn, W.A., Vane, F.M., Bugge, C.J., Carter, D.E., Bressler, R., Ehmann, C.W., 1985. Pharmacokinetics of 14C-isotretinoin in healthy volunteers and volunteers with biliary T-tube drainage. Drug Metab. Dispos. 13, 327–332.

Cotler, S., Bugge, C.J., Colburn, W.A., 1983. Role of gut contents, intestinal wall, and liver on the first pass metabolism and absolute bioavailability of isotretinoin in the dog. Drug Metab. Dispos. 11, 458–462.

Davis, M.E., Brewster, M.E., 2004. Cyclodextrin-based pharmaceutics: past, present and future. Nat. Rev. Drug Discov. 3, 1023–1035.

Frijlink, H.W., Franssen, E.J., Eissens, A.C., Oosting, R., Lerk, C.F., Meijer, D.K., 1991. The effects of cyclodextrins on the disposition of intravenously injected drugs in the rat. Pharm. Res. 8, 380–384.

Gollnick, H., Ehlert, R., Rinck, G., Orfanos, C.E., 1990. Retinoids: an overview of pharmacokinetics and therapeutic value. Meth. Enzymol. 190, 291–304.

Guchelaar, H.J., Wouda, S., Beukeveld, G.J., Mulder, N.H., Oosterhuis, J.W., 1992. Pharmacokinetics of parenteral 13-*cis*-retinoic acid formulations in rats. J. Pharm. Sci. 81, 432–435.

Hong, W.K., Endicott, J., Itri, L.M., Doos, W., Batsakis, J.G., Bell, R., Fofonoff, S., Byers, R., Atkinson, E.N., Vaughan, C., et al., 1986. 13-*cis*-Retinoic acid in the treatment of oral leukoplakia. N. Engl. J. Med. 315, 1501–1505.

Hong, W.K., Lippman, S.M., Itri, L.M., Karp, D.D., Lee, J.S., Byers, R.M., Schantz, S.P., Kramer, A.M., Lotan, R., Peters, L.J., et al., 1990. Prevention of second primary tumors with isotretinoin in squamous-cell carcinoma of the head and neck. N. Engl. J. Med. 323, 795–801.

Huselton, C.A., DeGrazia, F., Stahl, E., Liberato, D.J., 1996. A single dose pharmacokinetic study of 13-cis retinoic acid (isotretinoin, Ro 04-3780) in the pregnant New Zealand rabbit using intravenous infusion. Int. J. Pharm. 134, 157–165.

Kraemer, K.H., DiGiovanna, J.J., Moshell, A.N., Tarone, R.E., Peck, G.L., 1988. Prevention of skin cancer in xeroderma pigmentosum with the use of oral isotretinoin. N. Engl. J. Med. 318, 1633–1637.

Lin, J.H., Lu, A.Y., 1997. Role of pharmacokinetics and metabolism in drug discovery and development. Pharmacol. Rev. 49, 403–449.

Lin, H.S., Chan, S.Y., Low, K.S., Shoon, M.L., Ho, P.C., 2000a. Kinetic study of a 2-hydroxypropyl-beta-cyclodextrin-based formulation of all-*trans*-retinoic acid in Sprague–Dawley rats after oral or intravenous administration. J. Pharm. Sci. 89, 260–267.

Lin, H.S., Chean, C.S., Ng, Y.Y., Chan, S.Y., Ho, P.C., 2000b. 2-Hydroxypropyl-beta-cyclodextrin increases aqueous solubility and photostability of all-*trans*-retinoic acid. J. Clin. Pharm. Ther. 25, 265–269.

Lin, H.S., Barua, A.B., Olson, J.A., Low, K.S., Chan, S.Y., Shoon, M.L., Ho, P.C., 2001. Pharmacokinetic study of all-*trans*-retinoyl-beta-D-glucuronide in Sprague–Dawley rats after single and multiple intravenous administration(s). J. Pharm. Sci. 90, 2023–2031.

Liu, S.S., Sandri, R., Tang-Liu, D.D., 1990. Systemic pharmacokinetics of acetylenic retinoids in rats. Drug Metab. Dispos. 18, 1071–1077.

Liu, X., Lin, H.S., Thenmozhiyal, J.C., Chan, S.Y., Ho, P.C., 2003. Inclusion of acitretin into cyclodextrins: phase solubility, photostability, and physicochemical characterization. J. Pharm. Sci. 92, 2449–2457.

Liu, X., Lin, H.S., Chan, S.Y., Ho, P.C., 2004. Biopharmaceutics of beta-cyclodextrin derivative-based formulations of acitretin in Sprague–Dawley rats. J. Pharm. Sci. 93, 805–815.

Matthay, K.K., Villablanca, J.G., Seeger, R.C., Stram, D.O., Harris, R.E., Ramsay, N.K., Swift, P., Shimada, H., Black, C.T., Brodeur, G.M., Gerbing, R.B., Reynolds, C.P., 1999. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-*cis*-retinoic acid. Children's Cancer Group. N. Engl. J. Med. 341, 1165–1173.

Meloche, S., Besner, J.G., 1986. Metabolism of isotretinoin. Biliary excretion of isotretinoin glucuronide in the rat. Drug Metab. Dispos. 14, 246–249.

Patel, L.H., Khoo, K.C., Olejnik, N., Colburn, W.A., 1982. Comparative pharmacokinetics of isotretinoin and its metabolite, 4-oxo-isotretinoin, in dogs. Drug. Metab. Dispos. 10, 284–285.

Piel, G., Evrard, B., Van Hees, T., Delattre, L., 1999. Comparison of the IV pharmacokinetics in sheep of miconazole–cyclodextrin solutions and a micellar solution. Int. J. Pharm. 180, 41–45.

Sandberg, J.A., Eckhoff, C., Nau, H., Slikker Jr., W., 1994. Pharmacokinetics of 13-*cis*-, all-*trans*-, 13-*cis*-4-oxo-, and all-*trans*-4-oxo retinoic acid after intravenous administration in the cynomolgus monkey. Drug Metab. Dispos. 22, 154–160.

Shin, D.M., Glisson, B.S., Khuri, F.R., Clifford, J.L., Clayman, G., Benner, S.E., Forastiere, A.A., Ginsberg, L., Liu, D., Lee, J.J., Myers, J., Goepfert, H., Lotan, R., Hong, W.K., Lippman, S.M., 2002. Phase II and biologic study of interferon alfa, retinoic acid, and cisplatin in advanced squamous skin cancer. J. Clin. Oncol. 20, 364–370.

Stella, V.J., Rao, V.M., Zannou, E.A., Zia, V.V., 1999. Mechanisms of drug release from cyclodextrin complexes. Adv. Drug Deliv. Rev. 36, 3–16.

Tang, G.W., Russell, R.M., 1990. 13-*cis*-Retinoic acid is an endogenous compound in human serum. J. Lipid Res. 31, 175–182.

Wang, C.C., Campbell, S., Furner, R.L., Hill, D.L., 1980. Disposition of all-*trans*- and 13-*cis*-retinoic acids and *n*-hydroxyethylretinamide in mice after intravenous administration. Drug Metab. Dispos. 8, 8–11.

Ward, A., Brogden, R.N., Heel, R.C., Speight, T.M., Avery, G.S., 1984. Isotretinoin. A review of its pharmacological properties and therapeutic efficacy in acne and other skin disorders. Drugs 28, 6–37.

Yap, K.L., Liu, X., Thenmozhiyal, J.C., Ho, P.C., 2005. Characterization of the 13-*cis*-retinoic acid/cyclodextrin inclusion complexes by phase solubility, photostability, physicochemical and computational analysis. Eur. J. Pharm. Sci. 25, 49–56.