ASIAN JOURNAL OF PHARMACEUTICAL SCIENCES 12 (2017) 187–192

Available online at www.sciencedirect.com

ScienceDirect

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

Original Research Paper

Inclusion complex of tamibarotene with hydroxypropyl-β-cyclodextrin: Preparation, characterization, *in-vitro* and *in-vivo* evaluation

Ying Yang [1], *Jinhua Gao* [1], *Xiaoyu Ma*, *Guihua Huang* *

School of Pharmaceutical Sciences, Shandong University, 44 Wenhua Xi Road, Ji'nan, 250012, China

ARTICLE INFO

Article history:
Received 4 July 2016
Received in revised form 18 August 2016
Accepted 30 August 2016
Available online 21 September 2016

Keywords:
Tamibarotene
Hydroxypropyl-β-cyclodextrin
Solubility
Bio-availability

ABSTRACT

The goal of this study was to improve the solubility and oral bioavailability of tamibarotene by complexing it with hydroxypropyl-β-cyclodextrin (HP-β-CD). The inclusion complex of tamibarotene with hydroxypropyl-β-cyclodextrin (Am80-HP-β-CD) was prepared through a freeze-drying method at the mole ratio of 1:1 (Am80: HP-β-CD). Fourier transform infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC) indicated the formation of Am80-HP-β-CD. *In vitro* dissolution studies showed that the solubility and dissolution percentage of Am80-HP-β-CD was improved substantially compared to Am80. An improved dissolution with approximately 97% drug release in 3 min was observed, in comparison with Am80 with approximately 60% release in 45 min. *In vivo* studies indicated that the $AUC_{0-\infty}$ has increased 2.79 times and the C_{max} 4.37 times after the formation of inclusion complex. The decrease of t_{max} indicated the Am80-HP-β-CD inclusion complex can be absorbed into blood faster. In short, the solubility and bio-availability of Am80 has notably increased with the complexation of HP-β-CD. Therefore, using the inclusion technique is a promising method to improve the solubility of insoluble drugs.

1. Introduction

Tamibarotene, 4-[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl) carbamoyl] benzoic acid (Am80, the formula shown in Fig. 1), is a synthetic retinoid drug mainly used for relapsed or refractory acute promyelocytic leukemia (APL). Tamibarotene can be optionally combined with retinoic acid receptor α [1]; this combination recovers the normal function of primary marrow cells (PML) and the retinoic acid receptor gene leukemia α (RARα) by releasing the fusion of PML/RARα gene, which promotes the differentiation of promyelocytic cells, so that a therapeutic effect for APL can be achieved. Compared with other retinoid used for the treatment of APL, Am80 has a higher efficacy, produces less adverse reactions [2], and hardly has any drug resistance [3]. In addition, Am80 is less reactive to light, heat, oxidation, and other

* *Corresponding author.* School of Pharmaceutical Sciences, Shandong University, 44 Wenhua Xi Road, Ji'nan, 250012, China. Fax: +86 0531 88382548.
E-mail address: hgh2003@gmail.com (G. Huang).
[1] These authors contributed equally to this work.
Peer review under responsibility of Shenyang Pharmaceutical University.
http://dx.doi.org/10.1016/j.ajps.2016.08.009

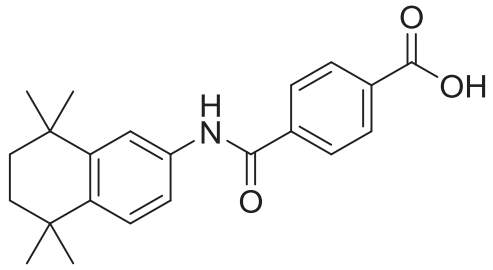

Fig. 1 – Tamibarotene.

physical and chemical factors. It is especially suitable for relapsed or refractory APL patients who are resistant to all trans retinoic acid (ATRA) [4]. In 2005, tamibarotene tablet was first approved for use against acute promyelocytic leukemia in Japan, with the trade name Amnolake and specifications of 2 mg. As deeper research, tamibarotene is being investigated for various disease, studies have shown that tamibarotene can be used for multiple myeloma [5], Crohn's disease [5], chronic obstructive pulmonary disease [6], liver cancer [7–10], solid tumors [5] and Alzheimer [3].

However, tamibarotene has a poor solubility; when administrated orally, the first-pass effect is obvious, the bioavailability is relatively low (50%–70%) and the absorption is poor. In addition, Am80 has no injection available because of its poor water solubility. Therefore, improving the solubility of Am80 and increasing its bioavailability for clinical use are problems need to be solved urgently.

Several methods can be used to enhance the solubility of insoluble drugs, such as the use of suitable solubilizers, making poorly soluble drugs into soluble prodrugs, the use of nano technology and inclusion technique, etc. Among them, inclusion technique can significantly improve the stability and solubility of insoluble drugs, so as to improve its bioavailability [3,5]. The preparation of an inclusion complex is simpler and requires less additive materials, making it more suitable for commercial production. A dozen cyclodextrin-containing drugs have already been approved and marketed [11]. Furthermore, inclusion complex can be used as an intermediate of various dosage forms. Therefore, this study intends to adopt the inclusion technology to prepare a water-soluble tamibarotene inclusion complex with improved bioavailability.

Cyclodextrin and its derivatives are widely used as pharmaceutical materials for preparing inclusion complexes due to its non-toxicity, high biodegradability and various other advantages [11]. They can be used to improve the water-solubility, chemical stability and bioavailability of insoluble drugs, as well as reducing drug toxicity [12]. Among three types of cyclodextrin, β-CD is the least soluble, making it the easiest to be crystallized from water. Thus, β-CD is the most commonly used cyclodextrin. HP-β-CD is the ether derivative of β-CD. Compared with β-CD, HP-β-CD is more soluble and less toxic [13]. When administered via injection, HP-β-CD has almost no stimulation to muscle and mucosal. It has been approved by the United States Food and Drug Administration (FDA) to be used as a drug solubilizing agent and penetration enhancer. Thus, HP-β-CD has great potential applied in pharmaceutical excipients [14].

The aim of this study was to prepare a tamibarotene hydroxypropyl-β-cyclodextrin inclusion complex (Am80-HP-β-CD) through a freeze-drying method so as to improve the solubility and bioavailability of tamibarotene. To observe the formation of the inclusion complex, Am80-HP-β-CD was characterized by differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FT-IR). Next, the *in-vitro* dissolution characteristics of Am80-HP-β-CD and Am80 were verified with a dissolution method. Furthermore, the pharmacokinetics characteristics and the improvements in bioavailability were verified through the *in-vivo* experiments performed on rats. These results suggest that HP-β-CD will be potentially useful in the delivery of water-insoluble anticancer agents such as tamibarotene.

2. Materials and methods

2.1. *Materials*

Am80 was obtained from Dalian Meilun Biological Technology Co., Ltd. (China). HP-β-CD was obtained from Shandong Xinda Technology Co., Ltd. (China), with average molecular weight Mw ~ 1410, and theory molar substitution of 4.7. Ethanol and HPLC grade methanol were obtained from Tianjin Kermel Chemical Reagent Co., Ltd. (China). All other reagents and solvents were of analytical grade. Wistar rats (female or male) weighing 200 ± 20 g were supplied by the Medical Animal Test Center of Shandong University. The animal experiment protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Shandong University.

2.2. *Methods*

2.2.1. *Preparation of Am80 -HP-β-CD inclusion complex*

The Am80-HP-β-CD inclusion complex was prepared via a freeze-drying method. The Am80 was first dissolved in ethanol (3 ml), and then dispersed the Am80 solution into HP-β-CD aqueous solution at the molar ratio of 1:1. The obtained suspension was then stirred continually for 4 h at room temperature. The ethanol was then entirely removed by rotary evaporation for 15 min at 65 °C, –0.08 MPa and then 10 ml distilled water was added and the solution was filtrated with a 0.45 μm microporous membrane. The solution was then cooled to room temperature and freeze-dried for 24 h. Finally, the inclusion complex was obtained.

2.2.2. *Characterization of Am80-HP-β-CD inclusion complex*

2.2.2.1. Appearance of Am80-HP-β-CD. The prepared Am80-HP-β-CD was placed in a dry Cillin bottle, and then observed its appearance.

2.2.2.2. Differential scanning calorimetry (DSC). The thermal characteristics of the Am80, HP-β-CD physical mixture and the Am80-HP-β-CD inclusion complex were determined using a differential scanning calorimeter (DSC 204, Netzsch, Germany). Samples were accurately weighed in an aluminum pan, with

an empty aluminum pan used as reference. The samples were then heated over a temperature range of 35–500 °C, at the heating rate of 10 °C/min under flowing nitrogen gas.

2.2.2.3. *Fourier transform infrared spectroscopy (FT-IR)*. The FT-IR spectra of Am80, HP-β-CD, physical mixture, and the Am80-HP-β-CD inclusion complex were obtained using a Fourier transform infrared spectrometer (Nicolet 6700 FT-IR Spectrometer, USA). Samples were prepared as KBr disks. The spectra were recorded from 4000 cm^{-1} to 400 cm^{-1} with 64 scans at the resolution of 4 cm^{-1}.

2.2.3. *Study of **in-vitro** dissolution*

The study of *in-vitro* dissolution of Am80-HP-β-CD (equivalent to 2 mg of Am80) and Am80 (2 mg) was conducted using USP dissolution apparatus II [Electrolab tablet dissolution tester (USP 24), India]. The samples were placed in 900 ml of dissolution medium (distilled water) at 37 °C, with a paddle rotating at 100 ± 1 rpm. 5 ml of samples were collected at various time intervals (3, 7, 10, 15, 20, 30, 45 min) and were replenished by an equal volume of fresh dissolution medium. The withdrawn samples were filtered through a 0.45 μm membrane filter, diluted properly, and then analyzed for Am80 content with UV at 280 nm. The results were presented as mean ± SD of triplicate experiments.

2.2.4. *Pharmacokinetic studies*

2.2.4.1. *HPLC conditions*. Concentration of tamibarotene in plasma was determined by HPLC. The HPLC equipment was a Shimadzu LC-10A system (Shimadzu, Kyoto, Japan) consisting of an LC-10AT HPLC pump and a SPD-10A UV-VIS detector. Data processing was performed with LC Solution software. Drug analysis was conducted on a Diamonsil TM-C18 column (4.6 mm × 150 mm, Dikma Technologies, China). The mobile phase was consisted of methanol, deionized water, and 5% glacial acetic acid (84:16, v/v) at the flow rate of 1.0 ml/min, and the injection volume was 20 μl. The detection wavelength was set to 280 nm.

2.2.4.2. *Sample preparation*. Collected 200 μl blood sample into the centrifuge tubes which were washed with heparin sodium. 300 μl acetonitrile was added and the mixture was vortexed for 30 sec. Next, the mixture was centrifuged for 10 min at 12,000 rpm, and the supernatant layer was filtered through a 0.22 μm filter. Finally, 20 μl of the filtered solution was injected into the HPLC instrument and the samples were analyzed by the HPLC method mentioned above.

2.2.4.3. *Method validation*. A stock solution of Am80 (40 μg/ml) was prepared in mobile phase. According to the sample preparation method, Am80 standard curve samples (0.1, 0.2, 0.4, 1.0, 2.0, 4.0, 10.0 μg/ml) were prepared by mixing a set of Am80 solution with blank plasma. The standard curve samples were then injected into HPLC system, and the peak area ratio of Am80 versus concentration was calculated. To evaluate the accuracy and precision of the method, low (0.1 μg/ml), medium (1.0 μg/ml), and high (10.0 μg/ml) concentration of samples were prepared.

2.2.4.4. *Pharmacokinetic study*. The study of *in-vivo* bioavailability was carried out on six adult Wistar rats (female or male) weighing 200 ± 20 g. The rats used for this study were housed individually under normal conditions and fasted overnight before the experiment with free access to water. Six rats were randomly assigned into two groups. One group was given tamibarotene solution through intragastric administration, while the other was given Am80-HP-β-CD inclusion complexes, both with dosage of 20 mg/kg. At specified time intervals (0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 24 h after intragastric administration), 300 μl blood samples was collected from jugular venous sinus. Then the blood samples were processed according to 2.2.4.2.

2.2.4.5. *Data analysis and statistics*. The plasma concentration versus time curves of the Am80 solution and Am80-HP-β-CD inclusion complex were plotted. Pharmacokinetic parameters were calculated by DAS 2.0 software. The corresponding sampling time (t_{max}) and the maximum peak concentration (C_{max}) were directly obtained from the curves.

3. Results and discussion

3.1. *Characterization*

3.1.1. *Appearance of Am80-HP-β-CD*

The freeze-dried powder of Am80-HP-β-CD was shown in Fig. 2. The powder was white and smooth. It is also soluble in distilled water. The aqueous solution is quite stable, it can be kept at 4 °C for at least one month.

3.1.2. *Differential scanning calorimetry (DSC)*

Change of sample temperature in the DSC test occurred due to a phase transition, or endothermic and exothermic effect of the samples. Generally speaking, the phase transition and dehydrogenation reduction will produce an endothermic effect of reducing dehydrogenation, while crystallization and oxidation will produce an exothermic effect. As shown in Fig. 3, tamibarotene has a typically sharp endothermic peak at 231 °C,

Fig. 2 – Appearance of Am80-HP-β-CD.

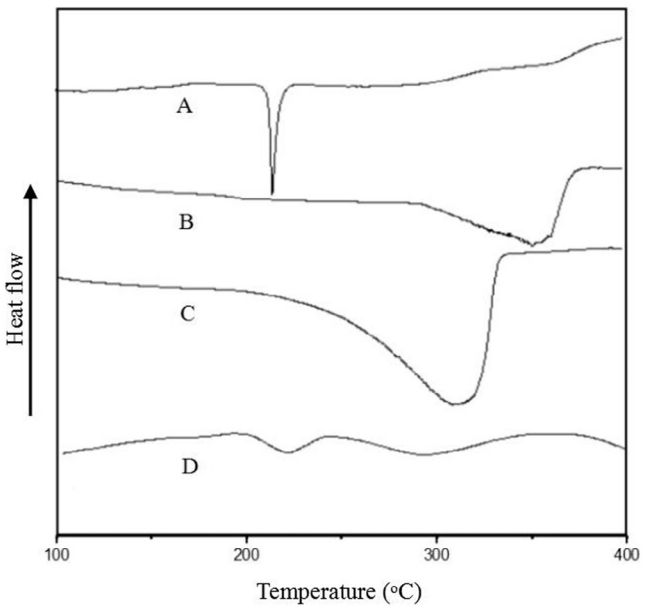

Fig. 3 – Results of DSC of A(tamibarotene), B (HP-β-CD), C (Am80-HP-β-CD) and D (the physical mixture).

HP-β-CD has a large characteristic absorption peak at about 360 °C; the absorption peak of the physical mixture of HP-β-CD and tamibarotene is slightly different from that of the superimposed peaks of tamibarotene and HP-β-CD. The interactions of Am80 and HP-β-CD may potentially be the causes. The Am80-HP-β-CD inclusion complex has formed a new absorption peak at 320 °C, without the typical peaks at around 231 °C (Am80) and 360 °C (HP-β-CD). This is caused by significantly weakened crystallization degree of tamibarotene when it formed an inclusion complex with HP-β-CD, which stops the thermal analysis from detecting the endothermic peak of the tamibarotene crystals (231 °C). Comparing the absorption peak of tamibarotene (231 °C) and HP-β-CD (360 °C), we can conclude that the Am80-HP-β-CD inclusion complex has been synthesized successfully.

3.1.3. *Fourier transform infrared spectroscopy (FT-IR)*

The FT-IR spectra of Am80, HP-β-CD, Am80-HP-β-CD inclusion complex, and their physical mixture are shown in Fig. 4. The spectrum of Am80 mainly consisted of major functional groups including phenyl, amide bond, carboxyl and carbonyl. The absorption bands attribute as the following: stretching vibration (3342.98 cm^{-1}) of O-H, stretching vibration (1697.80 cm^{-1}) of carbonyl, phenyl stretching vibration (3050 cm^{-1}) of ═CH and stretching vibration (1607.51 cm^{-1}, 1503.24 cm^{-1}, 1457.98 cm^{-1}) of C═C, aliphatic chain asymmetrical stretching vibration (2926.74 cm^{-1}, 2860.07 cm^{-1}) of —CH_2, and aliphatic chain stretching vibration (2964.75 cm^{-1}) of —CH_3. The spectrum of HP-β-CD mainly displayed the prominent absorption bands at 3405.98 cm^{-1} for O—H stretching vibration, 1645.70 cm^{-1} for H—O—H bending vibration, and 2926.05 cm^{-1} for —CH_2 asymmetrical stretching vibration. The spectrum of the physical mixture was evidently the superposition of the absorption spectra of Am80 and HP-β-CD. However, for the Am80-HP-β-CD inclusion complex, the typical absorption band of Am80 at 1697.80 cm^{-1} of carbonyl stretching vibration and phenyl stretching vibration (around 3050 cm^{-1}) of ═CH disappeared completely. These changes are relatable to HP-β-CD cavities of similar size, benzene, and cyclohexane group of Am80, inferring that the benzene and cyclohexane group of Am80 entered the HP-β-CD cavity, causing Am80 to be entrapped in the cavity of HP-β-CD.

3.2. *In vitro dissolution study*

The dissolution profiles of the Am80 solution and Am80-HP-β-CD inclusion complex were shown in Fig. 5. The cumulative dissolution percentage of Am80-HP-β-CD inclusion complex was

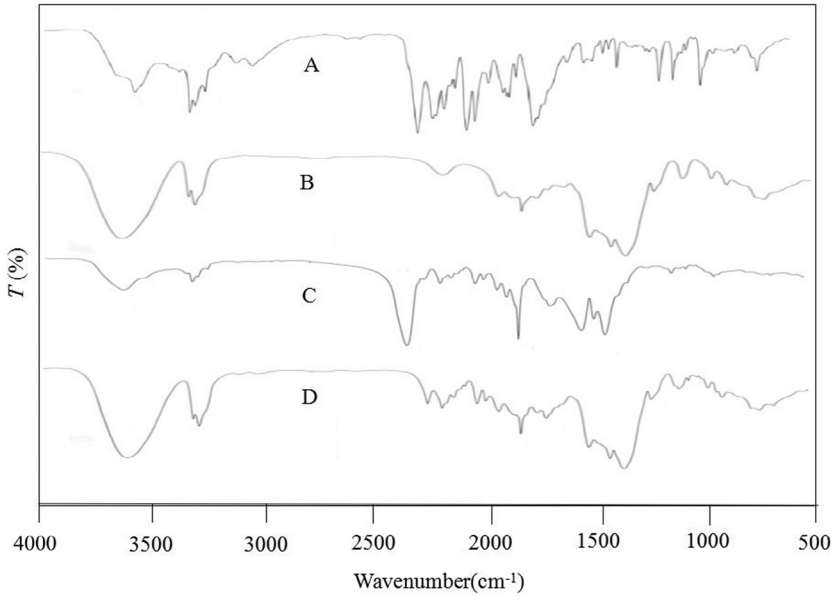

Fig. 4 – Spectrum of A(Am80), B (HP-β-CD), C (Am80-HP-β-CD) and D (the physical mixture).

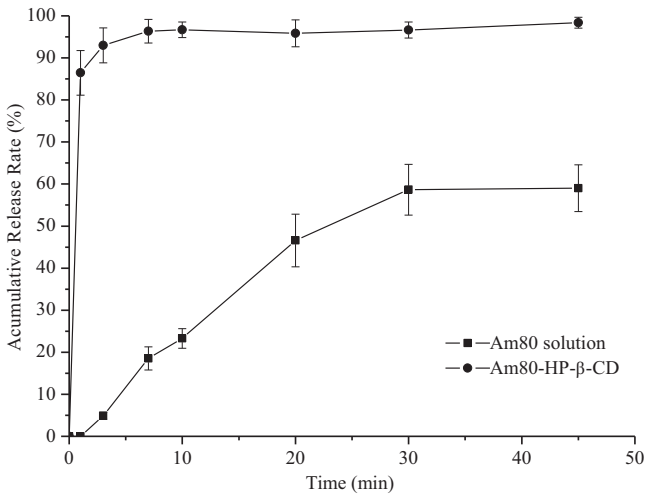

Fig. 5 – *In vitro* drug release kinetics of Am80 solution and Am80-HP-β-CD inclusion complex.
Data were expressed as mean values (±SD, n = 3).

significantly higher than that of Am80. As for Am80-HP-β-CD, the cumulative dissolution percentage was over 97% in the first 3 min, and stayed stable after 3 min, while for the Am80, the cumulative dissolution percentage was only 4.8% in the first 3 min, after 45 min, the cumulative dissolution percentage reached about 60% and stayed stable. The dissolution speed and the dissolution percentage of Am80-HP-β-CD were significantly higher than that of Am80. Thus, it can be concluded that the inclusion complex can significantly improve the dissolution efficiency. The cause could be that the Am80 crystal disappeared after the formation of Am80-HP-β-CD inclusion complex.

3.3. *In vivo studies*

3.3.1. *Analysis method validation results*

The typical chromatograms of Am80, blank plasma and plasma contained Am80 were shown in Fig. 6. The retention time for Am80 was about 7 min. It was shown that the endogenous components of plasma have no interference with Am80. The standard curve showed a good linearity within the range of 0.1~9.6 μg/ml. Typical mean linear equation of standard curves for Am80 was A = 26.352C-0.5541, r = 0.999, where A represented the peak area of Am80 and C was the concentration of Am80. The RSD values of inter-day and intra-day precision at three concentrations were less than 5% and 10%. The RSD values of the extraction yields of Am80 at three different concentration levels were less than 15%. The results showed that the method had good accuracy and precision. The extraction recovery would satisfy the requirements of the pharmacokinetic study.

3.3.2. *Pharmacokinetics and bioavailability study*

Mean plasma concentration versus time profiles of Am80-HP-β-CD inclusion complex and Am80 were illustrated in Fig. 7. Pharmacokinetic parameters were calculated by using the DAS 2.0 software; the results were shown in Table 1. By comparing the results of the two formulations, Am80-HP-β-CD inclusion

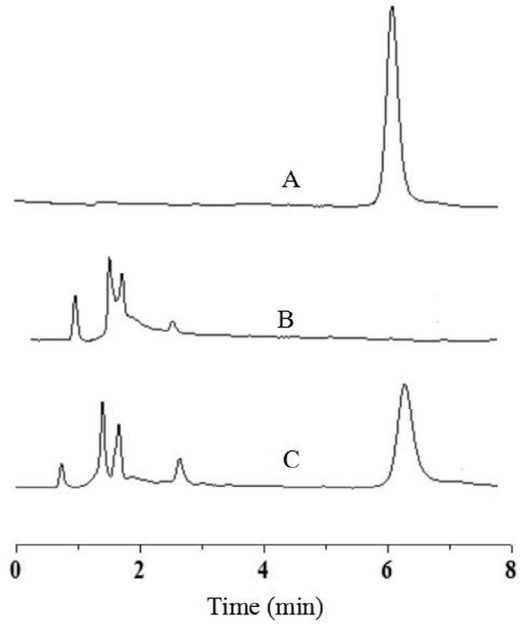

Fig. 6 – Typical chromatograms of A (Am80), B (blank plasma) and C (plasma contained Am80).

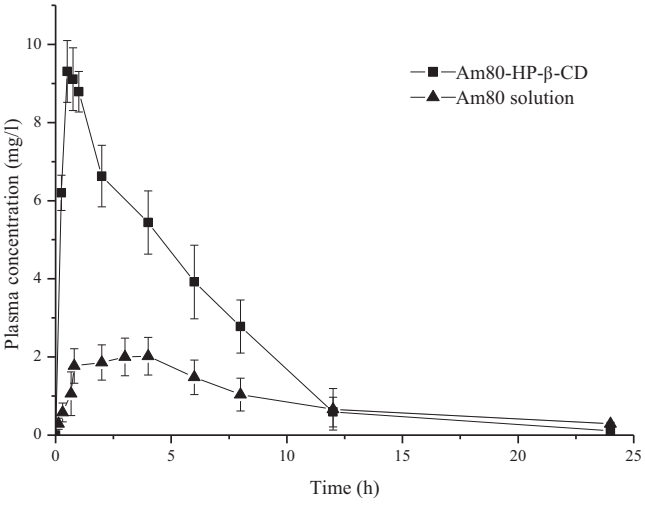

Fig. 7 – Plasma concentration (mg/l) after oral administration of Am80 and Am80-HP-β-CD inclusion complex.
Data were expressed as mean values (±SD, n = 3).

Table 1 – Pharmacokinetic parameters.

Parameters	Am80 solution	Am80-HP-β-CD
$AUC_{0-\infty}$ (mg/l · h)	17.08 ± 8.55	64.76 ± 4.73
CL(l/h/kg)	1.17 ± 0.29	0.31 ± 0.02
$MRT_{0-\infty}$ (h)	9.53 ± 0.82	4.77 ± 0.36
T_{max} (h)	4.00 ± 0.00	0.50 ± 0.00
C_{max} (mg/l)	1.77 ± 0.46	9.51 ± 0.33

complex had a significant decrease at t_{max}, MRT and Cl/F, and an increase at C_{max} and $AUC_{0-\infty}$.

To be specific, the $AUC_{0-\infty}$ of Am80-HP-β-CD inclusion complex was about 3.79 times compared with that of the Am80 after administration. Meanwhile, the C_{max} of Am80-HP-β-CD inclusion complex was about 5.37 times compared with the Am80. The increase of $AUC_{0-\infty}$ and C_{max} indicated the improvement of bioavailability. Evidently, the relative oral bioavailability of Am80-HP-β-CD inclusion complex increased about 2.79 times compared with Am80. Moreover, the t_{max} of Am80-HP-β-CD decreased 7 times compared with Am80, the decrease of t_{max} indicated Am80 can be absorbed into blood faster, which may be caused by the increase of solubility after the formation of the inclusion complex. The result of the *in vivo* studies was consistent with the *in vitro* studies. These results proved that the Am80-HP-β-CD inclusion complex could significantly enhance the oral bioavailability of various medications.

The poor absorption of Am80 may result from its low solubility and dissolution rate. The improvement of oral bioavailability for Am80 could be ascribed to the increase of its dissolution and water-solubility after Am80 entrapped into the HP-β-CD cavity.

4. Conclusion

In this study, the Am80-HP-β-CD inclusion complex was successfully prepared through a freeze-drying method at the mole ratio of 1:1. The DSC and FT-IR characteristics have proved the formation of the Am80-HP-β-CD inclusion complex. The in-vitro dissolution showed the dissolution efficiency of Am80-HP-β-CD inclusion complex was significantly higher than that of Am80. An improved dissolution with approximately 97% drug release in 3 min was observed, in comparison with Am80 with approximately 60% release in 45 min. *In vivo* studies indicated that the $AUC_{0-\infty}$ has increased 2.79 times and the C_{max} 4.37 times after the formation of inclusion complex. Further, the decrease of Cl/F and t_{max} indicated the Am80-HP-β-CD inclusion complex can be absorbed into blood faster as well as being cleaned out slower. In short, the bio-availability of Am80 was notably increased by complexing it with HP-β-CD.

Acknowledgments

The authors would like to express gratitude to the School of Pharmaceutical Science at Shandong University for providing the required infrastructure to carry out the research and the Drug Analysis and Testing Center at Shandong University for assistance with the FT-IR analysis. The authors would also like to thank Guanxuan (Jason) Zhai from the University of Waterloo for correcting the language.

REFERENCES

[1] Jin Y, Wang LY, Liu DX. Tamibarotene modulates the local immune response in experimental periodontitis. Int Immunopharmacol 2014;23:537–545.
[2] Miwako I, Kagechika H. Tamibarotene. Drugs Today (Barc) 2007;43:563–568.
[3] Mario J, Natascia M, Katalin E, et al. Comparative analysis of binary and ternary cyclodextrin complexes with econazole nitrate in solution and in solid state. J Pharm Biomed Anal 2014;91:81–91.
[4] Hiroshi F, Madoka N, Naoko Y, et al. Tamibarotene: a candidate retinoid drug for Alzheimer's disease. Biol Pharm Bull 2012;35:1206–1212.
[5] Liu M, Chen AJ, Wang Y, et al. Improved solubility and stability of 7-hydroxy-4-methylcoumarin at different temperatures and pH values through complexation with sulfobutyl ether-b-cyclodextrin. Food Chem 2015;168:270–275.
[6] Sakai H, Horiguchi M, Ozawa C, et al. Pulmonary administration of Am80 regenerates collapsed alveoli. J Control Release 2014;196:154–160.
[7] Kanai F, Obi S, Fujiyama S, et al. An open-label phase I/II study of tamibarotene in patients with advanced hepatocellular carcinoma. Hepatol Int 2013;8:94–103.
[8] Okusaka T, Ueno H, Ikeda M, et al. Phase I and pharmacokinetic clinical trial of oral administration of the acyclic retinoid NIK-333. Hepatol Res 2011;41:542–552.
[9] Fu J, Zhang JY, Wang FS. Tamibarotene: a new hope for therapeutic efficacy in hepatocellular carcinoma patients. Hepatol Int 2014;8:1–3.
[10] Shimizu M, Takai K, Moriwaki H. Strategy and mechanism for the prevention of hepatocellular carcinoma: phosphorylated retinoid X receptor α is a critical target for hepatocellular carcinoma chemoprevention. Cancer Sci 2009;100:369–374.
[11] Jozsef S. Utilization of cyclodextrins in industrial products and processes. J Mater Chem 1997;7:575–587.
[12] Larsen KL. Large cyclodextrins. J Incl Phenom Macrocycl Chem 2002;43:1–13.
[13] Gould S, Scott RC. 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD): a toxicology review. Food Chem Toxicol 2005;43:1451–1459.
[14] Lajos S, Jozsef S. Highly soluble cyclodextrin derivatives: chemistry, properties, and trends in development. Adv Drug Deliv Rev 1999;36:17–28.