Improvement of the Antitumor Activity of Poorly Soluble Sapacitabine (CS-682) by Using Soluplus® as a Surfactant

Tohru Obata,*,a Yuka Suzuki,a Noriko Ogawa,b Ippei Kurimoto,b Hiromitsu Yamamoto,b Tadahide Furuno,c Takuma Sasaki,a and Motohiro Tanakaa

aDepartment of Bioorganic Chemistry, School of Pharmacy, Aichi Gakuin University; bDepartment of Pharmaceutical Engineering, School of Pharmacy, Aichi Gakuin University; and cDepartment of Analytical Chemistry and Biophysics, School of Pharmacy, Aichi Gakuin University; 1–100 Kusumoto, Chikusa, Nagoya 464–8650, Japan.

Received November 14, 2013; accepted February 26, 2014

Sapacitabine (CS-682 or CYC682; 1-[2-cyano-2-deoxy-β-D-arabino-pentafuranosyl] N4-palmitoyl cytosine), a novel antitumor 2'-deoxycytidine analogue, shows a marked reduction in the water solubility because of the fatty acid side chain on the N4 group of the cytosine moiety. Poor water solubility is one of the important reasons why sapacitabine does not exert maximum antitumor activity. Therefore, we attempted to improve the water solubility of sapacitabine using a novel surfactant, Soluplus®, which consisted of a polyvinyl caprolactam–polyvinyl acetate–polyethylene glycol graft copolymer. In this study, we examined whether Soluplus® increased the water solubility and an antitumor activity of sapacitabine. The cytotoxicity of Soluplus® alone was lower than that of Tween 80 and Kolliphor® α-tocopherylpolyethylene glycol 1000 succinate (TPGS). The water solubility and the chemosensitivity of sapacitabine against several tumor cell lines to sapacitabine markedly increased upon using Soluplus®. In addition, the potential of Soluplus® including sapacitabine in increasing the antitumor activity was compared with sapacitabine alone in vivo. Although the total dose in the experimental period was considerably lower than the effective dose of sapacitabine alone, the life span of mice treated with sapacitabine containing 40 mg/mL Soluplus® increased by 150%. If Soluplus® was used as the solubilizing agent in clinical trials of sapacitabine, a low administration dose was appeared to require, and thus side effects might be prevented.

Key words sapacitabine; Soluplus®; antitumor; solubility; 1-[2-cyano-2-deoxy-β-D-arabino-pentafuranosyl]-cytosine (CNDAC)

MATERIALS AND METHODS

Chemicals Sapacitabine was kindly provided by Daiichi-Sankyo (Tokyo, Japan). Soluplus® and Kolliphor® TPGS (α-tocopherylpolyethylene glycol 1000 succinate) were kindly provided by BASF Japan (Tokyo, Japan). The structures of sapacitabine and Soluplus® are shown in Fig. 1. Tween 80 was purchased from Kishida Chemical (Osaka, Japan). Other reagents were purchased special grade reagents.

Preparation of Sapacitabine with Soluplus® Excess sacitabine (10 mg) was added into various concentrations of Soluplus® solvent (5 mL), and the suspensions were vortex mixed. After the solutions were shaken for 140 rpm at 37°C for 48 h, they were filtered using a 0.20 µm filter. The concentration of sapacitabine in the clear solution was quantified using HPLC. Briefly, the Soluplus® solution used to solubilize sapacitabine was evaporated and was then re-dissolved in acetonitrile. The HPLC conditions reported in a previous study were as follows: column, YMC Pack ODS-A 150 x 4.6 mm internal diameter (i.d.) (YMC, Kyoto, Japan); mobile phase, acetonitrile:0.5% acetic acid=4:1 v/v; temperature, 40°C; detector, UV; wavelength, 249 nm; flow rate, 1.0 mL/min; and injection volume, 20 µL. The concentration of sapacitabine was calculated using a standard curve. Sapacitabine without Soluplus® as a control was suspended in water and this suspension was used in vitro and in vivo experiments.

Tumor Cells Human fibrosarcoma cells HT-1080, human colon cancer cells DLD-1, human breast cancer cells MCF-7, and murine melanoma cells B16-F10 were purchased from the

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: tobata@dpc.agu.ac.jp © 2014 The Pharmaceutical Society of Japan
American Type Culture Collection (ATCC, Manassas, VA, U.S.A.). Human gastric cancer cells MKN-45 and murine leukemia cells P388 were obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan) and Japanese Foundation for Cancer Research (Tokyo, Japan), respectively. These cell lines were maintained at 37°C and 5% CO₂ in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin (Life Technologies, Carlsbad, CA, U.S.A.). For in vivo studies, P388 cells were maintained by weekly intraperitoneal (i.p.) passage to female DBA/2 mice (Charles River Japan, Kanagawa, Japan) in a specific pathogen-free area.

In Vitro Chemosensitivity The inhibitory effects of the drugs on the growth of tumor cells were examined using a colorimetric assay involving 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Briefly, 180 µL aliquots of an exponentially growing cell suspension (2000 cells/180 µL/well) were incubated with 20 µL of various concentrations of drugs diluted with water. After exposure to the drugs for 72 h, 20 µL of MTT solution (3 mg/mL) was added to each well, and the cell cultures were further incubated at 37°C for 4 h. After removal of the medium, the formed formazan crystals were dissolved in 200 µL of dimethyl sulfoxide. The absorbance of each well was measured at 570 nm using a microplate reader (MTP-800AFC; CORONA Electric, Hitachinaka, Japan), and the inhibition ratio (IR) was calculated using the following formula: IR (%) = \(1 - \frac{T}{C}\) × 100, where \(C\) is the mean of optical densities of the control group and \(T\) is that of the treatment.
group. The IC$_{50}$ value was defined as the concentration of the drug required to induce a 50% reduction in growth relative to that of the control. The IC$_{50}$ value was determined by a graphical correlation of the dose–response curve with at least three drug concentration points.

Antitumor Activity against P388 Mouse Leukemia Cells

P388 cells (1×10$^6$ cells) were i.p. implanted in 5-week-old female CDF1 mice (n=6 or 7) (Charles River Japan) on day 0, and each drug was serially administered i.p. on days 1–10. The ratio of survival days in treated mice (T) versus that in control mice (C) administrated with saline was calculated. Body weight and other appearance changes of mice were observed every day.

RESULTS

Cytotoxicity of Surfactants

We used some surfactants as solubilizing agents to dissolve the poorly soluble antitumor drug sapacitabine. First, we determined the cytotoxicity of the surfactants alone to cells that did not contain sapacitabine (Fig. 2). Soluplus$^\circledR$ showed no cytotoxicity even at high concentrations in HT-1080 and DLD-1 cell lines, and the ratio of inhibition of cell growth by Soluplus$^\circledR$ was less than 10%. Both cell lines showed high sensitivity to solutions containing the surfactants Tween 80 and Kolliphor$^\circledR$ TPGS, and the IC$_{50}$ values were 170 and 15 µg/mL, respectively. Additionally, no significant differences were observed among all surfactants in both cell lines.

Improvement of Solubility by Surfactants

Sapacitabine is highly lipophilic because of the palmitoyl group as a long side chain that protects the amino group of cytosine base. In order to improve the low solubility in water, three surfactants were attempted. All surfactants tested in a preliminary experiment showed an increase in the solubility of sapacitabine in water (data not shown). Since, surfactants such as Tween 80 and Kolliphor$^\circledR$ TPGS alone were cytotoxic to tumor cells, we examined only Soluplus$^\circledR$ as a surfactant to avoid an impairment against cell and to regard the safety. We determined the potential of Soluplus$^\circledR$ in increasing the water solubility of sapacitabine (Fig. 3). Sapacitabine was poorly water soluble, and its concentration in water could not be detected in our HPLC detection system (<0.02 µg/mL). Although the water solubility of sapacitabine was estimated to be 0.48 µg/mL by using the software Advanced Chemistry Development (ACD/Labs, Toronto, Canada), the actual water solubility of sapacitabine was extremely low. However, Soluplus$^\circledR$ was able to dissolve sapacitabine into water because of its amphiphilic molecular characteristics. When Soluplus$^\circledR$ was used as a surfactant, the water solubility showed a dose-dependent and marked increase in the water solubility of sapacitabine.

Increase in in vitro Antitumor Activity

An in vitro chemosensitivity was examined to determine whether the increase in water solubility of sapacitabine was related to its antitumor activity (Fig. 4). Sapacitabine alone, which was suspended in water without Soluplus$^\circledR$, showed enough antitumor effects, and its IC$_{50}$ value was 3.52 and 4.89 µg/mL in HT-1080 and DLD-1 cells, respectively (Table 1). The chemosensitivity to sapacitabine was increased by using Soluplus$^\circledR$ containing sapacitabine. Compared to sapacitabine alone, sapacitabine with Soluplus$^\circledR$ showed a marked increase in cytotoxicity in HT-1080 and DLD-1 cell lines. The IC$_{50}$ values were enhanced from 3 to 10 times and most efficient Soluplus$^\circledR$ dose was 80mg/mL in both cell lines. The effect of sapacitabine with Soluplus$^\circledR$ was also observed in other human (gastric tumor MKN-45 and breast tumor MCF-7) and mouse (melanoma B16-F10 and Leukemia P388) cell lines; in particular, the inhibition ratio of sapacitabine with Soluplus$^\circledR$ in P388 cell line was more than 80% although no cytotoxicity of sapacitabine only at same concentration (Table 2). And the cytotoxicity of only Soluplus$^\circledR$ (ca. 4000 µg/mL) was not observed in among these cell lines.

---

Fig. 3. Solubility of Sapacitabine with Soluplus$^\circledR$

The concentration of sapacitabine is shown as an average and the standard deviation (S.D.) values in each Soluplus$^\circledR$ solutions. *Not detection (<0.02 µg/mL).

Fig. 4. In Vitro Chemosensitivity of Sapacitabine Dissolved Using Soluplus$^\circledR$

The chemosensitivity of sapacitabine with Soluplus$^\circledR$ was expressed as an inhibition ratio (%) in HT-1080 (upper) and DLD-1 (lower) cells. Closed circle (●) indicates sapacitabine alone without Soluplus$^\circledR$. Opened circle (○), closed square (■), opened square (□), and closed triangle (▲) indicate 20, 40, 60, and 80mg/mL Soluplus$^\circledR$ including sapacitabine.
In Vivo Antitumor Activity of Sapacitabine Containing Soluplus®

An in vivo experiment was performed using P388 cells, which showed high sensitivity of CNDAC which is the mother compound of sapacitabine in an in vitro experiment. We used 4 and 40 mg/mL of Soluplus® including sapacitabine in this experiment. The concentration of sapacitabine was 5.592 µg/mL in 4 mg/mL Soluplus® including sapacitabine, which was equivalent to 0.056 mg/kg. This dose of sapacitabine was very low for it to show antitumor activity. Previous studies indicated that i.p. administration of 50 mg/kg of sapacitabine, which was equivalent to 0.056 mg/kg. This dose showed no significant change in body weight change and did not show other notable side effects (Table 3). Administration of sapacitabine with 4 mg/mL Soluplus® showed slight the antitumor activity. However, 40 mg/mL of Soluplus® including sapacitabine significantly prolonged the life span of mice (T/C, 150%). The high dose of Soluplus® including sapacitabine was equivalent to 0.496 mg/kg of sapacitabine, which was considerably lower than the optimal dose of sapacitabine alone. An antitumor activity in same amount of sapacitabine without Soluplus® didn’t change compared with control group. Administration of 4 mg/mL Soluplus® only showed slight prolongation of life span, however there was some dispersion on this experiment. When the rejection test for the data was performed, the statistic significance disappeared.

DISCUSSION

Many solubilizing reagents and techniques have been developed for poorly soluble drugs and have been used in experimental and clinical settings. BASF has introduced a new polymeric solubilizer, Soluplus®, which is a graft copolymer composed of polyethylene glycol, polyvinylcaprolactam, and polyvinylacetate. Soluplus® easily forms colloidal micelles with good solubilization capacity for poorly soluble drugs because it has a lower critical micelle concentration than other surfactants. We used Soluplus® to improve the water solubility of sapacitabine, and thus increase its antitumor activity. We examined the cytotoxicity of Soluplus® alone, and the results showed that cytotoxicity of Soluplus® alone was lower than that of Tween 80 and Kolliphor® TPGS. Tween 80 is a well-known non-ionic surfactant and an emulsifier and has been often used in foods. Kolliphor® TPGS is a recently developed solubilization agent for vitamin E derivatives and has been reported to increase the antitumor activity of doxorubicin and docetaxel. As these two surfactants showed cytotoxicity in vitro, these probably show marked in vivo cytotoxicity. In contrast, Soluplus® was not cytotoxic even at high concentrations (4000 µg/mL). Therefore, Soluplus® was safe to be used in clinical application. Additionally, the water solubility of sapacitabine markedly increased by using Soluplus®. It was presumed that amount of cellular uptake in aqueous phase were increased. Further, the antitumor activity of sapacitabine increased using Soluplus® and the IC₅₀ value of sapacitabine with Soluplus® was less than 1/4 of that with sapacitabine alone in both HT-1080 and DLD-1 cell lines. The antitumor activity of sapacitabine with Soluplus® appeared to be a dose-dependent increase likewise the soluble amount.

Table 1. Comparison of IC₅₀ Values of Sapacitabine among Different Concentrations of Soluplus®

| Soluplus conc. | HT-1080 | DLD-1 |
|---------------|---------|-------|
| 0 mg/mL       | 3.52 ± 2.42 | 4.89 ± 2.90 |
| 20 mg/mL      | 0.59 ± 0.46 | 0.84 ± 0.47 |
| 40 mg/mL      | 0.68 ± 0.45* | 1.32 ± 1.05* |
| 60 mg/mL      | 0.52 ± 0.22 | 0.52 ± 0.22 |
| 80 mg/mL      | 0.44 ± 0.29* | 0.48 ± 0.20 |

* showed significant difference from sapacitabine alone without Soluplus® using Student’s t-test (<0.05).

Table 2. Inhibition Ratio of Sapacitabine with Soluplus® against Tumor Proliferation in among Several Tumor Cell Lines

|          | P388 | B16-F10 | HT-1080 | MCF-7 | MKN-45 | DLD-1 |
|----------|------|---------|---------|-------|--------|-------|
| Sapacitabine with Soluplus® only | 81.5 | 58.9 | 68.8 | 64.0 | 57.8 | 68.7 |
| Sapacitabine only | −8.0 | −7.4 | −6.0 | 1.1 | −2.1 | 12.7 |
| Soluplus® only | −23.8 | −4.7 | 29.2 | 6.4 | −26.8 | −38.7 |

a) The 4 mg/mL solution of Soluplus® including 4.64 µg/mL of sapacitabine was prepared. This experiment was used at the concentration of 0.1 µg/mL sapacitabine. b) 0.1 µg/mL of sapacitabine was suspended with water. c) High concentration (4000 µg/mL) of Soluplus® without sapacitabine was examined.

Table 3. Soluplus® Including Sapacitabine Improved the Antitumor Activity

|       | n  | Range of body weight change | Median survival days | T/C (%) |
|-------|----|-----------------------------|---------------------|--------|
| Control | 6  | −1.9−+1.3 | 10 (10–11) | 120** |
| 4 mg/mL Soluplus® only | 6  | −1.4−+0.9 | 12 (10–18) | 110 |
| 40 mg/mL Soluplus® only | 6  | −2.3−+1.6 | 11 (10–11) | 100 |
| 0.056 mg/kg sapacitabine only | 6  | −0.8−+2.3 | 10 (6–11) | 105 |
| 0.496 mg/kg sapacitabine only | 6  | −1.4−+1.0 | 10.5 (10–11) | 105 |
| 4 mg/mL Soluplus® including sapacitabine | 7  | −1.8−+1.4 | 12 (10–13) | 120** |
| 40 mg/mL Soluplus® including sapacitabine | 7  | −2.3−+1.2 | 15 (3–15) | 150* |

a) The change in the body weight of mice was obtained by subtracting the average weight on day 0 from that on each day. b) Parentheses showed survival range. c) 4 or 40 mg/mL Soluplus® included with 5.592 or 49.6 µg/mL of sapacitabine, respectively. They were converted to 0.056 or 0.496 mg/kg of sapacitabine, respectively. * * * showed significant difference from control using Mann–Whitney’s U-test (<0.05 and <0.01, respectively).
of sapacitabine was dependent on Soluplus® concentration. However the soluble sapacitabine amount in water didn’t completely coincide with the antitumor activity. Sapacitabine with Soluplus® showed an increase in the antitumor activity of sapacitabine even in an in vivo study using the most popular model for antitumor assay of P388 mouse leukemia tumor. Sapacitabine with 4 and 40 mg/mL Soluplus® was significantly prolonged the life span of mice. Interestingly, the dose of sapacitabine with Soluplus® administered in this study was very low. Although the total dose for the experimental period was markedly lower than the effective dose of sapacitabine alone, the group treated with sapacitabine with 40 mg/mL Soluplus® showed a 150% increase in life span. The concentration of sapacitabine in the above solution was 49.6 µg/mL, which corresponded to a dose of 0.496 mg/kg. A previous study showed that greater amounts of sapacitabine were required for excellent antitumor activity, and its dose was estimated to be at least 100 mg/kg/experiment.1,4) The dose used in this study was less than 1/20 of the required amounts, which indicated a clear increase in the antitumor activity of sapacitabine using Soluplus®. Additionally, the group treated with Soluplus® alone and that treated with Soluplus® including sapacitabine showed no abnormal body weight changes and serious side effects throughout the experimental period. Sapacitabine with Soluplus® was increased antitumor activity both in vitro and in vivo study. Detailed mechanism was not clear but Soluplus® at high concentrations was considered to interfere with the culture medium as a solvent, and the micelles formed by Soluplus® may cause nonspecific binding to protein in the culture medium. Another possibility was speculated that high concentration of Soluplus® might reduce drug transport into tumor cellular in order to form the large size micelle of sapacitabine. Thus, our results indicated that Soluplus® was a safe and useful solubilizing reagent for sapacitabine in in vivo studies and in a clinical setting.

CONCLUSION

Sapacitabine is undergoing clinical trials in U.S. against different types of leukemia such as acute myelogenous leukemia (AML) and myelodysplastics syndrome (MDS).6) The limiting factors of sapacitabine were hematologic toxicities (mainly neutropenia) and gastrointestinal symptoms. The limiting factors of sapacitabine were hematologic toxicities and nucleosides and nucleotides. 14(1). Chemical stability of a new antitumor nucleoside, 2’-C-cyano-2’-deoxy-β-D-arabinofuranosylcytosine in alkaline medium: formation of 2’-C-cyano-2’-deoxy-β-D-arabinofuranosylcytosine and its antitumor activity. J. Med. Chem., 38, 3391–3397 (1995).

Acknowledgments This study was partially supported by the research Grant from the Institute of Pharmaceutical Life Sciences, Aichi Gakuin University. The in vivo animal experiment was approved by the ethics committee of Aichi Gakuin University.

REFERENCES

1) Hanaoka K, Suzuki M, Kobayashi T, Tanzawa F, Tanaka K, Shibayama T, Miura S, Ikeda T, Isabuchi H, Nakagawa A, Mitsuhashi Y, Hisaoka M, Kaneko M, Tomida A, Wataya Y, Nomura T, Sasaki T, Matsuda A, Tsurow T, Kurakata S. Antitumor activity and novel DNA-self-strand-breaking mechanism of CNDAC (1-(2-C-cyano-2-deoxy-β-D-arabinofuranosyl)cytosine) and its N4-palmitoyl derivative (CS-682). Int. J. Cancer, 82, 226–236 (1999).

2) Serova M, Galmarini CM, Ghoul K, Benthadi K, Green SR, Chiao J, Fairev S, Cvetkovic E, Le Tourneau C, Calvo F, Raymond E. Antiproliferative effects of sapacitabine (CYC682), a novel 2’-deoxycytidine-derivative, in human cancer cells. Br. J. Cancer, 97, 628–636 (2007).

3) Liu XJ, Nowak B, Wang YQ, Plunkett W, Sapacitabine, the prodrug of CNDAC, is a nucleoside analog with a unique action mechanism of inducing DNA strand breaks. Chin. J. Cancer, 31, 373–380 (2012).

4) Azuma A, Hanaoka K, Kurihara A, Kobayashi T, Miyauchi S, Kamo N, Tanaka M, Sasaki T, Matsuda A. Nucleosides and nucleotides. 14(1). Chemical stability of a new antitumor nucleoside, 2’-C-cyano-2’-deoxy-β-D-arabinofuranosylcytosine in alkaline medium: formation of 2’-C-cyano-2’-deoxy-β-D-arabinofuranosylcytosine and its antitumor activity. J. Med. Chem., 38, 3391–3397 (1995).

5) Matsuda A, Sasaki T. Antitumor activity of sugar-modified cytosine nucleosides. Cancer Sci., 95, 105–111 (2004).

6) Liu X, Kantarjian H, Plunkett W. Sapacitabine for cancer. Expert Opin. Investig. Drugs, 21, 541–555 (2012).

7) Kantarjian H, Garcia-Manero G, O’Brien S, Faderl S, Ravandi F, Westwood R, Green SR, Chiao JH, Boone PA, Cortes J, Plunkett W. Phase I clinical and pharmacokinetic study of oral sapacitabine in patients with acute leukemia and myelodysplastic syndrome. J. Clin. Oncol., 28, 285–291 (2010).

8) Bayes M. Gateways to clinical trials. Methods Find. Exp. Clin. Pharmacol., 29, 153–173 (2007).

9) Delaunoy T, Burch PA, Reid JM, Camoriano JK, Kobayash T, Braich TA, Kaur JS, Rubin J, Erlichman C. A phase I clinical and pharmacokinetic study of CS-682 administered orally in advanced malignant solid tumors. Invest. New Drugs, 24, 327–333 (2006).

10) Gilbert J, Carducci MA, Baker SD, Dees EC, Donchew R. A Phase I study of the oral antimitabolite, CS-682, administered once daily 5 d per week in patients with refractory solid tumor malignancies. Invest. New Drugs, 24, 499–508 (2006).

11) Jagan S, Paganessi LA, Frank RR, Venugopal P, Larson M, Christopher KW 2nd. Bone marrow and peripheral blood AML cells are highly sensitive to CNDAC, the active form of sapacitabine. Adv. Hematol., 2012, 72683 (2012).

12) Kantarjian H, Faderl S, Garcia-Manero G, Luger S, Venugopal P, Maness L, Wetzler M, Coulter S, Stock W, Claxton D, Goldberg St., Arienino M, Strickland SA, Seiter K, Schiller G, Jabbour E, Chiao J, Plunkett W. Oral sapacitabine for the treatment of acute myeloid leukemia in elderly patients: a randomised phase 2 study. Lancet Oncol., 13, 1096–1104 (2012).

13) Russell NH. Improving outcomes for elderly patients with AML. Lancet Oncol., 13, 1065–1066 (2012).

14) Zhu X, Ma Y, Liu D. Novel agents and regimens for acute myeloid leukemia: 2009 ASH annual meeting highlights. J. Hematol. Oncol., 3, 17–27 (2010).

15) Hardung DD, Ali S. Combining HME & Solubilization: Soluplus®—The Solid Solution. Drug Delivery Technology, 10, 20–27 (2010).

16) Alam MA, Ali R, Al-Jenobbi FL, Al-Mohizea AM. Solid dispersions: a strategy for poorly aqueous soluble drugs and technology updates. Expert Opin. Drug Deliv., 9, 1419–1440 (2012).

17) Linn M, Collnot EM, Djuric D, Hempel K, Fabian E, Kolter K, Lehr CM. Soluplus® as an effective absorption enhancer of poorly soluble drugs in vitro and in vivo. Eur. J. Pharm. Sci., 45, 336–343 (2012).
18) Thakral NK, Ray AR, Bar-Shalom D, Eriksson AH, Majumdar DK. Soluplus-solubilized citrated camptothecin—a potential drug delivery strategy in colon cancer. *AAPS PharmSciTech*, 13, 59–66 (2012).

19) Yu H, Xia D, Zhu Q, Zhu C, Chen D, Gan Y. Supersaturated polymeric micelles for oral cyclosporine A delivery. *Eur. J. Pharm. Biopharm.*, 85 (3 Pt B), 1325–1336 (2013).

20) Hughey JR, Keen JM, Brough C, Saeger S, McGinity JW. Thermal processing of a poorly water-soluble drug substance exhibiting a high melting point: the utility of KinetiSol® dispersing. *Int. J. Pharm.*, 419, 222–230 (2011).

21) Liu X, Lu M, Guo Z, Huang L, Feng X, Wu C. Improving the chemical stability of amorphous solid dispersion with cocrystal technique by hot melt extrusion. *Pharm. Res.*, 29, 806–817 (2012).

22) Nagy ZK, Balogh A, Vajna B, Farkas A, Patyi G, Kramarics A, Marosi G. Comparison of electrospun and extruded Soluplus®-based solid dosage forms of improved dissolution. *J. Pharm. Sci.*, 101, 322–332 (2012).

23) Cao N, Feng SS. Doxorubicin conjugated to d-alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS): conjugation chemistry, characterization, *in vitro* and *in vivo* evaluation. *Biomaterials*, 29, 3856–3865 (2008).

24) Feng SS, Mei L, Anitha P, Gan CW, Zhou W. Poly(lactide)–vitamin E derivative–montmorillonite nanoparticle formulations for the oral delivery of docetaxel. *Biomaterials*, 30, 3297–3306 (2009).

25) Yu Y, Tan S, Zhao S, Zhuang X, Song Q, Wang Y, Zhou Q, Zhang Z. Antitumor activity of docetaxel-loaded polymeric nanoparticles fabricated by Shirasu porous glass membrane-emulsification technique. *Int. J. Nanomedicine*, 8, 2641–2652 (2013).