Synthesis, formulation and in vitro evaluation of a novel microtubule destabilizer, SMART-100

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ABSTRACT

A novel microtubule destabilizer, substituted methoxynbenzoyl-ary-thiazole (SMART)-100, was synthesized, which showed good anticancer activity in HepG2 cells. SMART-100 was able to circumvent multidrug resistance (MDR) and effectively inhibited the growth of cell lines that overexpress P-glycoprotein (P-gp). SMART-100 inhibited P-gp activity, which may be responsible for its ability to overcome MDR. Since SMART-100 is poorly soluble in water, it was formulated in polyethylene-b-poly[(R)-lactide] (PEG-PLA) micelles. The solubility of SMART-100 was increased by more than 1.1×105 folds. SMART-100 loaded PEG-PLA micelles could effectively inhibit HepG2 cell growth and arrest cell cycle progression at G2/M phase, followed by appearance of a sub-G1 phase, which is indicative of cell apoptosis. Increased Caspase-3 activity was also observed when HepG2 cells were treated with SMART-100. The anticancer activity of SMART-100 loaded PEG-PLA micelles was also evaluated on luciferase expressing C4-2-Luc cell lines by IVIS imaging. Our results suggest that SMART-100 has the potential to treat resistant cancers.

1. Introduction

Several microtubule-targeting drugs, including paclitaxel and docetaxel, have been developed for treating breast, prostate and non-small-cell lung cancers with great success [1–5]. However, their anticancer effects are diminished because of their intrinsic or acquired drug resistance, which involves the over-expression of P-glycoprotein (P-gp), multidrug resistance protein 7 (MRP7) and the βIII-tubulin isotype [6–9]. Therefore, the development of novel anticancer drugs that can circumvent MDR will contribute significantly to the progress of cancer chemotherapy.Ixabepilone (IXEMPRA®), an epothilone analog, has recently been approved the FDA to be used as a monotherapy or in combination with capecitabine for treating patients with metastatic or locally advanced breast cancer resistant to treatment with an anthracycline and a taxane, or whose cancer is taxane resistant and for whom further anthracycline therapy is contraindicated [10].

Recently, we have also have synthesized a series of substituted methoxybenzoyl-ary-thiazole (SMART) with good anticancer activity on different cancer cell types, including leukemia, non-small cell lung cancer, colon cancer, CNS cancer, renal cancer, melanoma, ovarian cancer, prostate cancer, and breast cancer [11]. The SMART compounds work as microtubule destabilizers by inhibiting tubulin polymerization. Compared with other microtubule-targeting drugs [9], SMART drugs are much easier to synthesize, which will reduce the manufacturing cost and thus make them more affordable to patients. Although SMART compounds demonstrated excellent anticancer bioactivities, their use was limited due to their poor intrinsic aqueous solubility. Two possible approaches could be used to address this problem. One is chemical modification, which may involve an extensive structure activity relationship (SAR) study. Another way is to improve the solubility of SMART drugs through formulation approaches. Conventionally, dimethyl sulfoxide (DMSO), Cremophor EL, polysorbate 80 (Twee80) or other surfactants are commonly used to solubilize hydrophobic drugs such as taxane. However, the use of these solubilizing agents is usually associated with liver and kidney toxicity, hemolysis, acute hypersensitivity reaction and peripheral neuropathies [12].

Biodegradable polymeric micelles have recently been developed as vehicles for hydrophobic drugs, since these micelles significantly increase the aqueous solubility of hydrophobic drugs and reduce their toxicity by avoiding the use of toxic co-solvents or solubilizing agents [13]. The improved safety profile makes it possible to administer drugs at a higher dose which may enhance their therapeutic efficacy while minimizing their side effects. Previously, we used a poly (ethylene)-b-poly[(R)-lactide] (PEG-PLA) polymeric micelles to solubilize two hydrophobic drugs such as bicalutamide and embelin, and at least 60-fold increase in drug aqueous solubility was observed [14]. Micelles prepared using PEG-PLA copolymer has PLA as a hydrophobic core, which is responsible for drug loading, and PEG as a hydrophilic...
shell to confer stealth properties and thus prevents recognition by the reticuloendothelial system (RES) [15]. Due to its small size of 20–60 nm and stealth properties, a passive tumor targeting using these micelles through enhanced permeability and retention (EPR) could be achieved [16].

In this study, the anticancer effect of SMART-100 on cancer cells and its ability to circumvent P-gp mediated drug resistance were determined. PEG-PLA micelles were used to improve the aqueous solubility of SMART-100. In addition, the in vitro anticancer activity of SMART-100 loaded micelles and its mechanism of action were determined.

2. Experimental methods

2.1. Materials

Formyl terminated PEG-PLA of 4500–5100 Da was purchased from Polymer Source (Montreal, Canada). HepG2 and C4-2 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA). Parent DU145 and paclitaxel-resistant DU145-TXR cells were provided by Professor Evan T. Keller of the University of Michigan. Caspase Glo™ 3 assay kit was purchased from Promega (Madison, WI). All other reagents were obtained from Sigma-Aldrich (St. Louis, MO).

2.2. Synthesis of SMART-100

SMART-100, (2-phenyl-thiazol-4-yl)-(3,4,5-trimethoxy-phenyl)-methanone, was synthesized as described [11]. Briefly, benzonitrile (40 mmol) was mixed with L-cysteine (45 mmol) in 100 ml of 1:1 MeOH/PH 6.4 phosphate buffer solution (PBS). The reaction was stirred at 40 °C for 3 days. The precipitate was removed by filtration, and methanol was removed using a rotary evaporator. 1 M hydrochloric acid was added to the remaining solution to adjust pH to 2 at 0 °C. The resulting precipitate was filtered to yield a white solid 2-phenyl-4,5-dihydrothiazole-4-carboxylic acid, which was used directly for the next step.

A mixture of 2-phenyl-4,5-dihydrothiazole-4-carboxylic acid (5 mmol), 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide (EDCI, 6 mmol) and hydroxobenzotriazole (HOBt, 5 mmol) in CH2Cl2 (50 ml) was stirred for 10 min. To this solution, N-methylmorpholine (NMM, 11 mmol) and HNCH3OCH3 HCl salt (5 mmol) were added and stirring continued at room temperature for 6–8 h. The reaction mixture was diluted with CH2Cl2 (100 ml) and sequentially washed with water, satd. NaHCO3, brine and dried over MgSO4. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound SMART-100.

2.3. Preparation and characterization micelles

2.3.1. Preparation of micelles

Drug loaded micelles were prepared by a film dispersion method. Briefly, various amounts of SMART-100 and PEG-PLA were dissolved in acetonitrile and a thin film was formed after removing solvent under reduced pressure. The resulting film was hydrated and sonicated. The resultant formulation was then centrifuged to remove residual free drug. The supernatant was then filtered using a 0.22 μm filter and used within 48 h.

2.3.2. Determination of drug solubility and loading efficiency

To determine water solubility of free drug, 1 mg of SMART-100 was suspended in 1 ml water and shake for 48 h at room temperature. The suspension was centrifuged at 10,000 rpm for 10 min and filtered on 0.22 μm filter. To determine the solubility of SMART-100 in micelles, 50 μl of SMART-100 loaded micelles solution was diluted with acetonitrile, and the drug concentration was measured by UV-spectrometer or reverse phase high performance liquid chromatography (RP-HPLC, Waters, Milford, MA) with UV detector at 292 nm using a reverse phase C18 column (250 mm 4.6 mm, Alltech, Deerfield, IL). The mobile phase was composed of 40:60 V/V water and acetonitrile. SMART-100 concentration was calculated by peak area according to the following calibration equation: $C = 188.24A - 593.64$ $(R^2 = 1$, detection limit: 2 ng/ml). The drug encapsulation efficiency of SMART-100 in polymeric micelles was calculated with the following equation:

$$\text{Drug Encapsulation Efficiency} \% = \frac{\text{Weight of drug in micelle}}{\text{Weight of drug originally fed}} \times 100\%.$$

Fold of drug solubility increase were calculated with the following equation:

$$\text{Fold of drug solubility increase} = \frac{\text{Drug solubility in micelles}}{\text{Drug solubility in water}}.$$

2.3.3. Measurement of particle size

The particle size distribution of micelles was determined by dynamic light scattering with Malvern Nano ZS. The intensity of scattered light was detected at 90°.

2.4. Propidium iodide staining and cell cycle analysis

Cells were cultured in a 24-well plate to 90% confluence and treated with SMART-100 (2.8 μM) for 24 h. Cells were trypsinized and fixed in 70% ice-cold ethanol. After washing with PBS, cell pellet was re-suspended in 5 μg/ml propidium iodide staining solution for 15 min at room temperature. Cell cycle distribution was measured by flow cytometry (Becton, Dickinson, NJ). Results from 10,000 fluorescent events were obtained for analysis.

2.5. MTT assay

Cells were seeded in 96-well plates at a density of 5000 cells per well. At the end of treatment, cell culture media was replaced by 100 μl medium with 0.5 mg/ml MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide and incubated for 1 h at 37 °C. After removing the media, 200 μl of DMSO was added into each well to dissolve the formazan crystals. The absorbance was measured in a microplate reader at a wavelength of 560 nm. Cell viability was expressed as the percentage of control group.
2.6. Calcein acetoxymethylester (Calcein AM) assay

Cells were seeded into a black wall clear bottom 96-well plate at a density of 20,000 cells per well one day before the experiment. After treating cells with various concentration of SMART-100 in 50 μl DPBS for 20 min at 37 °C, 50 μl Calcein AM (10 μM) in DPBS were added to each well and the plate was incubated at 37 °C for additional 20 min. Fluorescence intensity in each well were determined using a SpectraMax M2/M2e spectrophuorometer (Sunnyvale, CA) at the excitation wavelength of 494 nm and emission wavelength of 517 nm.

2.7. Determination of caspase-3 activity

Cells were seeded in a 48-well plate to reach 80% confluence before experiment. After treating the cells for 16 h, culture media was removed and 100 μl Caspase Glo 3 reagent was added to each well. After gently mixing the content in each well, the plate was incubated at room temperature for 60 min protected from light. Finally, 70 μl of the reaction solution was measured using a luminometer.

2.8. Establishing a luciferase expression cell line for IVIS imaging

For stable luciferase expression, C4-2 cells were seeded in a 6-well plate 24 h before being transfected with pRc/CMV2-Luc plasmid (from Dr. Shu Wang, National University of Singapore) for 18 h. Two days after transfection, cells were transferred into a T-25 cell culture flask and selected with 600 μg/ml G418. The resulting bioluminescent cells were maintained in media containing 300 μg/ml G418. These cells were then seeded from 120,000 to 100 cells into a black wall clear bottom 96-well plates. D-luciferin (150 μg/ml) in DPBS was added to each well at 2 min prior to imaging using an IVIS imaging system (Xenogen, Alameda, CA).

3. Results

3.1. Synthesis and characterization of SMART-100

SMART-100 was synthesized as described by Lu et al. [11] and illustrated in Fig. 1A. SMART-100 was characterized by 1H NMR (Fig. 1B), 13C NMR and ESI-MS. 1H NMR (300 MHz, CDCl3): δ 8.29 (s, 1 H), 8.03 (q, 2 H), 7.80 (s, 2 H), 7.49–7.47 (m, 3 H), 3.96 (s, 6 H), 3.97 (s, 3 H). 13C NMR (75 MHz, CDCl3): 185.1, 168.1, 167.0, 155.4, 152.7, 133.1, 131.9, 130.6, 129.1, 127.7, 126.8, 109.1, 60.9, and 56.2. MS (ESI) m/z 378.1 [M+Na]+.

3.2. Anti-cancer activity of SMART-100 on HepG2 cells

The anticancer activity of SMART-100 was determined in HepG2 cells with MTT assay. SMART-100 effectively inhibited HepG2 cell growth (Fig. 2), with an IC50 of around 0.56 μM. The effect was observed as early as 24 h post treatment and became more significant after 48 h post treatment. There is no significant difference between "0" group, which is the cells treated with 0.5% DMSO and "DMEM" group, which is the cells treated with DMEM cell culture medium. Dose response curve was of typical sigmoid shape, with steep linear response at the mid-range dose groups. While there was no measurable effect at low doses (≤0.06 nM), the dose response curve became less steep and finally reaches plateau at high dose groups.

3.3. Anti-cancer activity of SMART-100 in resistant prostate cancer cells

We also tested the anticancer effect of SMART-100 on DU145-TXR cell, a paclitaxel-resistant prostate cancer cell line with high P-gp activity and the parent DU145 cell, a paclitaxel-sensitive cell with low P-gp activity. Even though paclitaxel showed good activity on DU145 cells (IC50<50 nM), its efficacy was greatly reduced on DU145-TXR cell.

![Fig. 1. Synthesis and characterization of SMART-100. (A) Synthesis scheme of SMART-100. (B) 1H NMR spectrum of SMART-100.](image-url)
cells and killing only 20% of cells at a concentration of 1 µM (Fig. 3A). In contrast, SMART-100 showed equally good anticancer activity on both DU145 and DU145-TXR cells with IC50 around 70 nM (Fig. 3B). From Fig. 3B, we could see that SMART-100 at 1 µM inhibited 63.7% and 67.6% cancer cell growth for DU145 and DU145-TXR, respectively. 74.2% (DU145) and 74.9% (DU145-TXR) cancer cell growth inhibition was achieved by SMART-100 at concentration of 5.63 µM. 82.1% (DU145) and 83.8% (DU145-TXR) cancer cell growth inhibition was achieved by SMART-100 at concentration of 11.26 µM. 

Intracellular calcein levels in DU145 cells were much higher than those in DU145-TXR cells (Fig. 4A), indicating higher P-gp activities in DU145-TXR cells but not in DU145 cells. The treatment of verapamil, a P-gp inhibitor, significantly increased intracellular calcein levels in DU145-TXR cells in a dose dependent manner, while no changes in intracellular calcein levels were observed in DU145 cells. Treatment of DU145-TXR cells with SMART-100 also increased intracellular calcein levels, while treatment of paclitaxel showed no effect (Fig. 4B). This indicated that SMART-100 might also act as a P-gp inhibitor in addition to its anti-mitotic activity and thus overcome the drug resistance in DU145-TXR cells due to P-gp over-expression.

### 3.4. Micellar solubility of SMART-100

Although SMART-100 showed good anticancer activity, its application was limited due to its poor water solubility, which is less than 2 ng/ml. Therefore, PEG-PLA micelles were used to improve its solubility. A film dispersion method was used to prepare micelles [14]. The mean particle size of micelles was around 50 nm and no significant difference in particle size was observed in micelles with and without drug. These micelles were stable for at least 12 days without significant change in particle size.

When we increased the theoretical loading from 0.5% to 5% w/w, the drug solubility increased from 26.8 ± 0.1 µg/ml to 48.0 ± 0.3 µg/ml until theoretical loading reached 1% (Fig. 5A). The drug loading efficiency decreased significantly after the theoretical loading became larger than 1% (Fig. 5B). To further increase the drug solubility, the effect of polymer concentration on drug solubility was determined. The solubility of SMART-100 increased linearly from 48.0 ± 0.3 to 213.9 ± 8.1 µg/ml (2.4 × 10⁴ to 1.1 × 10⁵ folds as that of free SMART-100 in water), when the polymer concentration was increased from 5 mg/ml to 20 mg/ml (Fig. 5C). The drug loading efficiency was around 100% for all tested polymer concentration (Fig. 5D).

### 3.5. Anticancer effect of SMART-100 loaded PEG-PLA micelles

No obvious cytotoxicity was observed in blank PEG-PLA micelles group with a polymer concentration of as high as 1 mg/ml (Fig. 6). Significant anticancer effect was observed in both SMART-100 loaded PEG-PLA micelles and SMART-100 dissolved in DMSO and the anticancer activity increased with the dose. Even though the final
DMSO concentration used was 0.5%, which did not show any obvious toxicity on our experiment, no direct comparison can be made with the results of SMART-100 dissolved in DMSO, as DMSO is known to kill tumor and may not be suitable for in vivo applications.

3.6. Cell cycle perturbation

The treatment of SMART-100 formulated in both DMSO and PEG-PLA micelles caused cell cycle arrest in HepG2 cells, while no effect was observed after treating cells with blank PEG-PLA micelles or DMSO (Fig. 7). The treatment of SMART-100 formulations caused the cells in G0/G1 phase decreased from 49.18±1.59% in the control group to 21.03±0.54% in DMSO dissolved SMART-100 group and 19.24±0.89% in PEG-PLA micelles formulated SMART-100 group, respectively. While the cells in G2/M phase were increased from 29.59±1.55% in control group to 73.06±1.68% in DMSO dissolved SMART-100 group and 74.91±1.10% in PEG-PLA micelles formulated SMART-100 group, respectively. In addition, we also determined the effect of treatment time on cell cycle distribution (Fig. 7B). Cells were treated with SMART-100 loaded PEG-PLA micelles for 24, 48, and 72 h. There was no change in the percentage of cells in G0/G1 phase after treating the cells for prolonged time at 48 or 72 h. The percentage of the cells in G2/M phase was decreased from 74.91±1.1% at 24 h to 68.71±1.95% at 48 h and to 65.5±1.65% at 72 h. In addition, the cells in sub G1 phase were also increased from 0.29±0.21% at 24 h to 2.96±1.34% at 48 h and to 8.94±1.15% at 72 h. The increase of cell population in subG1 phase indicated that apoptotic cells were increased after SMART-100 treatment for prolonged time.

3.7. Caspase-3 activity

In the cell cycle analysis, we found that the treatment of SMART-100 resulted in increased cell population in subG1 phase, which is an indication of apoptosis. To confirm whether cell death induced by SMART-100 was associated with apoptosis, caspase-3 activity was measured after treating HepG2 cells with SMART-100. As shown in Fig. 8, no significant difference was observed between untreated cell and cells treated with DMSO or empty PEG-PLA micelles. The treatment of SMART-100 with both DMSO and PEG-PLA micelles at a concentration of 2.8 µM resulted in increased caspase-3 activity (p<0.05), which is comparable to those caused by paclitaxel with the same molar concentration (p<0.05). This result indicated that SMART-100 may cause cell apoptosis by the activation of caspase-3 activity. However, increase of caspase-3 activity in SMART-100 treated groups was relatively low compared with cells treated with staurosporine, a well-known caspase 3 activator (p<0.01).

3.8. IVIS imaging for assessing antitumor activity

We established a luciferase expressing C4-2 prostate cancer cell line for IVIS imaging (Fig. 9A). There was a good correlation between the cells number and bioluminescence signals captured by IVIS imaging (R²=0.9769, Fig. 9B), thus live cell number could be quantitatively determined by IVIS imaging. Treatment with SMART-100 resulted in significant reduction in the bioluminescence signal (Fig. 9C). However, the treatment with empty PEG-PLA micelles did not cause any change in bioluminescence signal. The viable cell number has also been quantitatively determined by measuring bioluminescence signal (Fig. 9D).
4. Discussion

The discovery of paclitaxel has resulted in significant progress for the treatment of several cancers including breast, ovarian, and non-small cell lung cancers. Paclitaxel works through stabilizing microtubules, and thus causing mitotic arrest and apoptosis. Despite the potent anticancer activity of paclitaxel, its clinical application is often undermined due to the occurrence of MDR. Cancer cells become...
resistant to anticancer drugs due to the change in cell membrane ATP-binding cassette (ABC) super family transporters such as P-gp, multiple drug resistance protein (MRP) and breast cancer resistance protein (ABCG2). Particularly, the over-expression of P-gp encoded by MDR1 gene is widely investigated and known to be responsible for the resistance to several anticancer agents such as paclitaxel and docetaxel.

Several strategies have been studied to overcome MDR mechanism including the use of novel drug delivery systems, co-administration of P-gp inhibitors, and the search of new compounds that could overcome MDR. Drug delivery systems such as HPMA copolymer drug conjugates [17], polymeric micelles [18], liposomes [19] and nanoparticles [20] have been successfully used to reduce the drug resistance by enhanced intracellular drug delivery and/or inhibition of P-gp activities. In addition to formulation approaches, scientists have also successfully discovered several new compounds to overcome MDR. Ixabepilone, for example, has been approved by the FDA as a drug for MDR breast cancer [21].

To test whether SMART-100 could overcome MDR, a paclitaxel-resistant cell line, DU145-TXR, was used in this study. DU145-TXR is resistant to paclitaxel as well as several other anticancer agents including estramustine phosphate, docetaxel, doxorubicin and vinblastine [22]. SMART-100 showed potent anticancer activities in both paclitaxel-sensitive (DU145) and paclitaxel-resistant (DU145-TXR) cancer cells (Fig. 3B). These results suggest that SMART-100 has the potential to be used for treating MDR cancers.

To further understand why SMART-100 could keep its anticancer activity in paclitaxel-resistant cells capable of overexpressing P-gp, Calcein acetoxymethylester (Calcein AM) assay was carried out to determine the effect of SMART-100 on P-gp functions. Calcein AM is a substrate for efflux activity of P-gp. In cells with high P-gp activity, Calcein-AM is constantly pumped out, preventing intracellular accumulation of fluorescent calcein. The inhibition of P-gp function by inhibitors such as verapamil results in reduced efflux of fluorescent calcein and thus increases intracellular fluorescence intensity. The change of fluorescence intensity could be quantitatively determined by fluorometer and used as an indicator for P-gp activities. The treatment of SMART-100 significantly increased the intracellular accumulation of calcein, which indicated that it might be an inhibitor of P-gp and prevented the efflux of drug out of P-gp overexpressing cells (Fig. 4). Therefore, inhibition of P-gp activity might be a mechanism of SMART-100 to overcome multidrug resistance.

Our previous study showed that SMART-100 works by targeting and destabilizing microtubules. As a microtubule destabilizer, its anti-proliferative effect is involved in the inhibition of tubulin polymerization [11]. The cell cycle analysis indicated that after treatment with SMART-100 the cells were arrested in G2/M phase (Fig. 7). Since the destabilization of microtubule dynamics will result in the cell cycle arrest at mitotic phase, cell cycle analysis experiment further confirmed that the anticancer mechanism of SMART-100 is through destabilizing microtubule. We also found that after SMART-100 treatment cells in sub-G1 phase were significantly increased.

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**Fig. 8.** Effect of SMART-100 on caspase-3 activity. After treatment with 2.8 μM SMART-100, paclitaxel or staurosporine for 16 h, caspase-3 activity was determined. Caspase-3 activities were expressed as the relative light unit (RLU) and normalized by cell viability. Drugs were dissolved in DMSO, unless otherwise stated. Results are the mean ± SE (n = 3). *p < 0.001, **p < 0.01, ***p < 0.05 compared with DMSO control. +p < 0.01 compared with blank PEG-PLA micelles. #p < 0.001, compared with staurosporine.

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**Fig. 9.** Determination of anticancer activity by IVIS imaging on C4-2 luciferase expressing cells. (A) In vitro bioluminescence of C4-2 human prostate tumor cell lines expressing luciferase. C4-2-luc cells were seeded from 120,000 to 100 cells per well and imaged after the addition of luciferin. Wells containing cells but no luciferin were served as negative controls. (B) Correlation between cell number and mean bioluminescence signal. (C) Effect of SMART-100 loaded PEG-PLA micelles on the inhibition of C4-2-Luc cell growth. Cells were treated with blank PEG-PLA micelles and SMART-100 loaded micelles for 4 days at the dose of 1 μM and the in vitro bioluminescence was determined by IVIS imaging. (D) The cell viability was quantitatively determined by bioluminescence reading and expressed as percentage of control group. *p<0.001, compared with blank PEG-PLA control.
The increase in cell population in sub-G1 phase indicated the induction of cell apoptosis by SMART-100 [23,24]. An increase in caspase 3 activity is an important marker for cell apoptosis. Treatment with SMART-100 increased caspase 3 activities in this study (Fig. 8), which indicated the occurrence of apoptosis. From the binding of microtubule destabilizer with the tubulin to apoptosis is a complicated intracellular process, which involves multiple signal pathways, including the checkpoint of mitotic spindle activation, the activation of cyclin-dependent kinases, and the JNK/SAPK [25]. However, these signal pathways are beyond the scope of this study.

PEG-PLA micelles have been successfully used as a Cremophor EL free formulation for paclitaxel (Genexol-PM), which is under multicenter phase II clinical trial [26]. Because of its superior performance as a delivery system for paclitaxel, we used PEG-PLA micelles to improve the solubility of SMART-100. Due to the high logP value (4.08) of SMART-100, its aqueous solubility is very low (below the detection limit, 2 ng/ml). However, the use of PEG-PLA micelles significantly increased its aqueous solubility. The maximum solubility reached to 213.9 ± 8.1 µg/ml, which increased SMART-100 solubility by at least 1 x 105 folds (Fig. 5). Polymeric micelles are known to enhance solubilization of hydrophobic compounds by accommodating them in their hydrophobic core of polymer. A critical parameters involves in the solubilization process is polymer–drug compatibility, which could be evaluated by solubility difference (Δ) and Flory–Huggins interaction parameter (Xhp) [27]. We have calculated the solubility parameters of SMART-100 and PLA hydrophobic core by Molecular Modeling Pro Software (ChemSW Inc., Fairfield, CA). The calculated parameters for PLA is 19.7 (id), 2.48 (sp) and 20.9 (ih), while those of SMART-100 is 20.3 (id), 6.5 (sp) and 9.2 (ih). Thus, the solubility difference (Δ) between PLA and SMART-100 is 12.4. Typically, a solubility difference value less than 5 is needed to observed good solubility [28]. Therefore, we plan to design and modify the hydrophobic core of the polymer to make it more compatible with SMART-100 and further increase the solubility of SMART-100.

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References


