Plasma, Tumor, and Tissue Disposition of STEALTH Liposomal CKD-602 (S-CKD602) and Nonliposomal CKD-602 in Mice Bearing A375 Human Melanoma Xenografts

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Abstract

Purpose: S-CKD602 is a STEALTH liposomal formulation of CKD-602, a camptothecin analogue. The cytotoxicity of camptothecin analogues is related to the duration of exposure in the tumor. STEALTH liposomal formulations contain lipid conjugated to methoxypolyethylene glycol and have been designed to prolong drug circulation time, increase tumor delivery, and improve the therapeutic index. For STEALTH liposomal formulations of anticancer agents to achieve antitumor effects, the active drug must be released into the tumor extracellular fluid (ECF).

Experimental Design: S-CKD602 at 1 mg/kg or nonliposomal CKD-602 at 30 mg/kg was administered once via tail vein to mice bearing A375 human melanoma xenografts. Mice (n = 3 per time point) were euthanized at 0.083 to 24 h, 48 h, and 72 h after S-CKD602 and from 0.083 to 24 h after nonliposomal CKD-602. Plasma samples were processed to measure encapsulated, released, and sum total (encapsulated plus released) CKD-602, and tumor and tissue samples were processed to measure sum total CKD-602. Microdialysis samples of tumor ECF were obtained from 0 to 2 h, 4 to 7 h, and 20 to 24 h after nonliposomal CKD-602 and from 0 to 2 h, 24 to 27 h, 48 to 51 h, and 72 to 75 h after S-CKD602. A liquid chromatography-mass spectrometry assay was used to measure the total (sum of lactone and hydroxyl acid) CKD-602. The area under the concentration-versus-time curves (AUC) from 0 to infinity and time >1 ng/mL in tumor were estimated.

Results: For S-CKD602, the CKD-602 sum total AUC in plasma and tumor and the CKD-602 AUC in tumor ECF were 201,929, 13,194, and 187 ng/mL h, respectively. For S-CKD602, 82% of CKD-602 remains encapsulated in plasma. For nonliposomal CKD-602, the CKD-602 AUC in plasma and the CKD-602 AUC in tumor ECF were 9,117, 11,661, and 639 ng/mL h, respectively. The duration of time the CKD-602 concentration was >1 ng/mL in tumor ECF after S-CKD602 and nonliposomal CKD-602 was >72 and ~20 h, respectively. For S-CKD602, the CKD-602 sum total exposure was 1.3-fold higher in fat as compared with muscle. The ratio of CKD-602 sum total exposure in fat to muscle was 3.8-fold higher after administration of S-CKD602 compared with nonliposomal CKD-602.

Conclusion: S-CKD602 provides pharmacokinetic advantages in plasma, tumor, and tumor ECF compared with nonliposomal CKD-602 at 1/30th of the dose, which is consistent with the improved antitumor efficacy of S-CKD602 in preclinical studies. The distribution of S-CKD602 is greater in fat compared with muscle whereas the distribution of nonliposomal CKD-602 is greater in muscle compared with fat. These results suggest that the body composition of a patient may affect the disposition of S-CKD602 and released CKD-602.

It is currently unclear why within a patient with solid tumors there can be a reduction in the size of some tumors whereas other tumors can progress during or after treatment, although the genetic composition of the tumors is similar (1–3). Such variable antitumor responses within a single patient may be associated with inherent differences in tumor vascularity, capillary permeability, and/or tumor interstitial pressure, which result in variable delivery of anticancer agents to different tumor sites (1–3). However, studies evaluating the intratumoral concentration of anticancer agents and factors affecting...
tumor exposure in preclinical models and patients are rare. It is logistically difficult to carry out the extensive studies required to evaluate the tumor disposition of anticancer agents and factors that determine the disposition in patients with solid tumors, especially in tumors that are not easily accessible (2–5). Thus, there is impending need to develop and implement techniques and methods to evaluate the disposition and exposure of anticancer agents within the tumor matrix.

S-CKD602 is a STEALTH liposomal formulation of CKD-602, a camptothecin analogue (6–8). S-CKD602 is currently in clinical development (7). The STEALTH liposomal formulation consists of phospholipids covalently bound to methoxypolyethylene glycol (mPEG) on the outside of the lipid bilayer. CKD-602 inhibits topoisomerase I, thereby preventing DNA replication causing apoptosis (6, 7, 9–11). Nonliposomal CKD-602 administered i.v. at 0.5 mg/m²/d for 5 consecutive days repeated every 21 days is approved in Korea for the treatment of newly diagnosed small-cell lung cancer and for relapsed ovarian cancer (9–11). In animal models, a 3- to 10-fold increase in therapeutic index was observed with S-CKD602 compared with nonliposomal CKD-602 (11). The cytotoxicity of camptothecin analogues has been reported to be related to the duration of time the concentration is above a critical threshold (12–15).

The development of STEALTH liposomes was based on the discovery that incorporation of mPEG-lipids into liposomes yields preparations with prolonged plasma exposure and superior tumor delivery compared with conventional liposomes composed of natural phospholipids. The disposition of the STEALTH liposome–encapsulated drug is dictated by characteristics of the liposome, such as size, surface charge, membrane lipid packing, steric stabilization, dose, and route of administration, all of which alter the plasma pharmacokinetic profile and tissue distribution of the drug (6, 16, 17). Once the drug is released from the liposome, the pharmacokinetic disposition will be the same as after administration of the nonliposomal formulation of the drug (6, 16, 17). Overall, the advantages of liposome-encapsulated drugs are increased solubility, selective delivery of entrapped drug to the site of action, prolonged duration of exposure of released active drug in the tumor extracellular fluid (ECF), improved therapeutic index, and potentially overcoming resistance associated with the regular anticancer agent (6, 16, 17). Liposomes extravasate through leaky capillary beds of tumors and lodge into the interstitial spaces among tumor cells, where they release the encapsulated drug (6, 16–18). For anticancer agents encapsulated in liposomes to be an effective treatment in patients with solid tumors, the active form of the anticancer agent must be released from the liposome into the tumor ECF and then penetrate into the cell (6, 18).

Microdialysis is an in vivo sampling technique used to study the pharmacokinetics and drug metabolism in the blood and ECF of various tissues and tumors (1, 4, 5, 19). The use of microdialysis methods to evaluate the disposition of anticancer agents in tumors is relatively new. Microdialysis is based on the diffusion of non–protein-bound drugs from interstitial fluid across the semipermeable membrane of the microdialysis probe. Microdialysis allows for repeated sampling of drugs in the ECF of tissues and tumors. The released and non–albumin-bound drug can be recovered due to the molecular cutoff of 20 kDa of the semipermeable membrane of the microdialysis probe. Microdialysis provides a means to obtain from tumor ECF samples from which a concentration-time profile can be determined within a single tumor. By microdialysis, we were previously unable to detect released platinum (Pt) in tumor ECF after administration of STEALTH liposomal cisplatin (SPI-077; ref. 18). The results of this study suggested that SPI-077 distributes into tumors but release less unbound Pt into tumor ECF and form fewer Pt-DNA adducts as compared with cisplatin, which was consistent with low antitumor activity of SPI-077 (18). Moreover, no study has reported detectable released drug in tumor ECF after administration of a liposomal or nanoparticle anticancer agent (6, 18).

The objectives of the current study were to evaluate the plasma, tissue, and tumor disposition of S-CKD602 and nonliposomal CKD-602 in female SCID mice bearing A375 human melanoma xenografts. New sample processing methods were developed to evaluate the encapsulated and released CKD-602 in plasma after administration of S-CKD602. Microdialysis was used to evaluate the release of CKD-602 from S-CKD602 in tumor ECF. The determination of the tumor ECF disposition of released CKD-602 was evaluated by microdialysis methods.

Materials and Methods

Mice. All mice were handled in accordance with the Guide to the Care and Use of Laboratory Animals (National Research Council, 1996), and studies were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh Medical Center. Mice (female C.B-17 SCID, 4–6 weeks of age, and specific pathogen free) were obtained from Taconic and were allowed to acclimate to the animal facilities at the University of Pittsburgh for 1 week before initiation of study. Mice were housed in microisolator cages and allowed I.S.DPRO autoclavable rodent chow (Harlan Tekla Diets) or I.S.DPRO RMH3000 irradiated rodent chow (PMI Nutrition International, Inc.) and mice received water ad libitum. Body weights and tumor size were measured twice weekly and clinical observations were made twice daily.

Tumor lines. The A375 human melanoma xenograft was used in our study based on previous antitumor activity of S-CKD602 in this xenograft line and the fact that melanoma tumors provide adequate tumor delivery and exposure of drug in tumor ECF for microdialysis recovery (11, 18). A375 human melanoma cell lines were obtained from the DCTD Tumor Repository and were mouse antigen production test negative. A375 cells were expanded in culture and injected (1 × 10⁷ per mouse) s.c. into passage mice. The A375 tumors were harvested when they were 1 to 2 g and were implanted as ~25-mg fragments s.c. on the right flank of SCID mice by aseptic techniques. Tumor volumes were calculated with the following formula: length × (width)² / 2, where length is the largest diameter and width is the smallest diameter perpendicular to the length (20). Pharmacokinetic and microdialysis studies were done when the tumors were ~1,000 to 1,500 mm³ (1.1–1.5 g) in size.

Formulation and administration. S-CKD602 is a STEALTH or pegylated liposomal formulation of CKD-602. The clinical formulation of S-CKD602 was used in this study (ALZA Corp.; ref. 7). S-CKD602 STEALTH liposomes are composed of the lipids distearoylphosphatidylcholine and distearoylphosphatidylethanolamine covalently bound to mPEG. The mean particle size of the S-CKD602 liposomes is ~100 nm. CKD-602 lactone is encapsulated in the aqueous core of the liposome with an encapsulation efficiency of >85%. The concentrations of S-CKD602 refer to actual doses of CKD-602. S-CKD602 was administered at 1 mg/kg i.v. push via tail vein over ~1 min. This dose is one half the maximum tolerated dose in mice (11). The dose of S-CKD602 administered was based on the maximum volume of drug allowed to be administered i.v. by our Institutional Animal Care and Use Committee.
Nonliposomal CKD-602 was administered at 30 mg/kg i.v. push via tail vein (11). This dose is approximately the maximum tolerated dose for a single dose of nonliposomal CKD-602 in mice. Nonliposomal CKD-602 was prepared at 3 mg/mL in 270 mmol/L mannitol and 0.4 mmol/L tartaric acid in a 5% dextrose solution at pH 3.6. The vehicle control for S-CKD602 and nonliposomal CKD-602 was 0.9% NaCl and 270 mmol/L mannitol and 0.4 mmol/L tartaric acid in a 5% dextrose solution at pH 3.6, respectively.

**Pharmacokinetic studies.** Due to limited sample volume, the pharmacokinetic and microdialysis studies were done in separate groups of mice. Pharmacokinetic studies of S-CKD602 were done after administration of vehicle and at 5 min, 0.25, 0.5, 1, 2, 4, 7, 16, 24, 48, and 72 h after administration. Pharmacokinetic studies of nonliposomal CKD-602 were done after administration of vehicle and at 5 min, 0.25, 0.5, 1, 2, 4, 7, 16, and 24 h after administration. For each pharmacokinetic study, mice (n = 3 per time point) were euthanized with carbon dioxide and heparinized blood samples (−0.8-1 mL) were collected by cardiac puncture. The blood samples were centrifuged at 12,000 × g for 4 min. After S-CKD602 administration, the plasma was processed to measure encapsulated, released, and sum total (encapsulated + released) CKD-602. For S-CKD602 and nonliposomal CKD-602, tumor, liver, kidney, spleen, brain, peritoneal cavity fat, and bicep femoris skeletal muscle samples were obtained for measurement of sum total drug. Pharmacokinetic studies evaluated the camptothecin total concentration of vehicle and at 5 min, 0.25, 0.5, 1, 2, 4, 7, 16, and 24 h after administration of S-CKD602 and nonliposomal CKD-602.

**Sample processing.** The plasma for the pharmacokinetic studies of S-CKD602 was immediately placed on ice but not frozen. Plasma samples of S-CKD602 cannot be frozen because freezing the plasma sample ruptures the liposome and thus prevents the differentiation between encapsulated and released drug. After administration of S-CKD602, sum total, encapsulated, and released CKD-602 concentrations in plasma were measured in separate aliquots from the same sample. The separation of liposomal encapsulated and released CKD-602 in plasma was accomplished by solid-phase separation. An aliquot of 100 μL plasma was mixed with internal standard (10 μL of D7-CKD-602, 5 μg/mL) and loaded onto a BondElut LRC SPE cartridge (Varian). The cartridge was preconditioned with 2-mL methanol and 2 mL 0.9% saline. Encapsulated CKD-602 was eluted and collected with 2 mL of 0.9% saline. The cartridge was then washed with an additional 5 mL of 0.9% saline to remove any remaining encapsulated CKD-602. Released CKD-602 and internal standard were eluted with 2-mL acetonitrile acidified with 0.1% formic acid.

The encapsulated CKD-602 samples in plasma were processed by taking the 2 mL 0.9% saline eluant and adding 10 μL of internal standard. Salt was removed and the sample was extracted by adding 2-mL methylene chloride and 2 mL 50 mmol/L ammonium acetate (pH 8.3). The sample was vortexed and centrifuged at 3,000 × g for 6 min at 5°C. The organic layer (bottom layer) was transferred to 10 × 75-mm borosilicate glass tubes and dried under nitrogen gas at 37°C. The dried residue was suspended in 100-μL methanol of mobile phase, transferred into autosampler vials, and centrifuged at 7,000 × g for 6 min at 5°C to remove particulates.

The 2 mL acidified acetonitrile aliquot containing released CKD-602 was dried under nitrogen gas at 37°C and suspended in 100 μL mobile phase. The sample processing of plasma for sum total CKD-602 was done by extracting 100 μL of plasma with 0.5 mL of acidified acetonitrile. The samples were vortexed, centrifuged at 20,000 × g for 6 min at 5°C, and decanted into 10 × 75 mm borosilicate glass tubes and dried under nitrogen and suspended in mobile phase. Plasma samples for nonliposomal CKD-602 were immediately frozen in liquid nitrogen and stored at −80°C until analyzed. The sample processing of plasma for CKD-602 was done as described above for sum total S-CKD602 and nonliposomal CKD-602.

Tumor and tissue samples for S-CKD602 and nonliposomal CKD-602 were weighed, snap frozen in liquid nitrogen, and stored at −80°C until analyzed. The sum total samples of CKD-602 in tumor and tissues were processed by homogenizing tissues in PBS (pH 7.0) at 1:3 (w/v). Aliquots of 100 μL homogenate were then transferred to micro-centrifuge tubes, mixed with 10 μL internal standard, and extracted with 500 μL acidified acetonitrile. The samples were centrifuged at 20,000 × g for 6 min at 5°C. The supernatants were decanted and dried under nitrogen at 37°C. The dried residue was suspended in 100 μL of mobile phase.

**Microdialysis studies of tumor ECF disposition.** Microdialysis studies were done to evaluate the tumor ECF disposition of nonliposomal CKD-602 and released CKD-602 from S-CKD602 (1, 4, 5, 19). After administration of S-CKD602, microdialysis studies (n = 3–4 mice per interval) were obtained every 20 min from 0 to 2 h and every 30 min from 24 to 27 h, 48 to 51 h, and 72 to 75 h. After administration of nonliposomal CKD-602, microdialysis studies (n = 3–4 mice per interval) were obtained every 20 min from 0 to 2 h and every 30 min from 4 to 7 h and 20 to 24 h. Microdialysis probe recovery was estimated by retrodialysis calibration from 0 to 2 h after administration of S-CKD602 and nonliposomal CKD-602 as previously described (19). At all other microdialysis sample intervals, probe recovery was estimated with camptothecin as a tracer agent. The concentration of camptothecin was constant during the microdialysis...
sampling. The estimated recovery of the CKD-602 in the tumor ECF after administration of nonliposomal CKD-602 and S-CKD602 was 0.44 ± 0.16% and 0.39 ± 0.15%, respectively. Tumor ECF samples of CKD-602 after administration of S-CKD602 and nonliposomal CKD-602 were processed by adding 10 μL of the internal standard. At the end of each microdialysis procedure, plasma was processed to measure encapsulated, released, and sum total S-CKD602. Tumor and tissue samples were also obtained and processed as described above to measure sum total CKD-602.

Analytic studies. A liquid chromatography-mass spectrometry assay was used to measure the camptothecin total (sum of lactone and hydroxy acid) forms of encapsulated, released, and sum total CKD-602 in plasma; sum total CKD-602 in tumor and tissues; and CKD-602 in tumor ECF after administration of S-CKD602. In addition, the liquid chromatography-mass spectrometry assay was used to measure CKD-602 in plasma, tumor, and tissues after administration of nonliposomal CKD-602. This liquid chromatography-mass spectrometry assay was modified from our previous assay for 9-nitrocamptothecin (20).

The high-performance liquid chromatography system consisted of a Finnigan Spectra Systems AS3000 autosampler and P4000 quaternary pump (ThermoFinnigan) with a Synergi Hydro-RP 80A (4 mm, 100 × 2 mm) analytical column (Phenomenex). The isocratic mobile phase consisted of 0.1% formic acid in methanol/water (35:85, v/v) and was pumped at 0.2 mL/min. Injection volume was 10 μL and the run time was 10 min. Column eluant was analyzed with a ThermoFinnigan aQa mass spectrometer (ThermoQuest) operating in electrospray positive mode electron ionization monitoring CKD-602 and the internal standard D7-CKD-602 at 434.1 and 441.2 m/z, respectively. The insert probe temperature was set at 400°C with 3 kV ion spray voltage and 20 V orifice voltage. Nitrogen gas flow was fixed at 75 p.s.i. at the tank head unit. The system was operated with ThermoFinnigan Xcaliber software. The CKD-602-to-internal standard ratio was calculated for standards by dividing the analyte peak area by that of the internal standard. Standard curves for CKD-602 were constructed by plotting the analyte-to-internal standard ratio versus the known concentration of the analyte in each standard. Standard curves were fit by linear regression with 1/y weighting and back calculation of CKD-602 concentrations. The lower limit of quantitation for CKD-602 in tumor ECF was 0.1 ng/mL. The lower limit of quantitation for sum total, encapsulated, and released CKD-602 in plasma was 1 ng/mL. The lower limit of quantitation for sum total CKD-602 in tumor and tissues was 1 ng/mL.

Pharmacokinetic analysis. Pharmacokinetic analysis was done using noncompartmental methods. The area under the concentration-versus-time curves for sum total, encapsulated, and released CKD-602 in plasma and sum total in tumor and tissues were estimated from 0 to infinity (AUC_{0-\infty}) using the log trapezoidal method. The maximum concentration (C_{max}), time of C_{max} (T_{max}), time of last concentration (T_{last}), and the time the concentration of CKD-602 was >1 ng/mL in tumor ECF were determined by visual inspection for all studies.

Results

Plasma and tissue sum total pharmacokinetic disposition. We compared the plasma, tissue, and tumor pharmacokinetic disposition of sum total CKD-602 after administration of nonliposomal CKD-602 and S-CKD602. The concentration-versus-time profile of sum total CKD-602 in plasma, tissue, and tumors after administration of nonliposomal CKD-602 is presented in Fig. 1A and B. The sum total pharmacokinetic parameters after administration of nonliposomal CKD-602 are presented in Table 1. After administration of nonliposomal CKD-602, the plasma concentration-versus-time profile of CKD-602 peaked at 0.083 h (5 min) after administration, had a biphasic elimination profile, and was no longer detectable after 16 h. The concentration-versus-time profiles of CKD-602 in all tissues were similar to the profile in plasma. The exposure of CKD-602 was higher in tumor compared with plasma and the other tissues from 7 to 24 h. Consistent with the distribution and elimination of other nonliposomal camptothecin analogues, the highest exposures of CKD-602 in tissues after administration of nonliposomal CKD-602 were in the liver and kidney. The overall distribution of nonliposomal CKD-602 was 3-fold greater in muscle compared with fat.

The concentration-versus-time profile of sum total CKD-602 in plasma, tissue, and tumors after administration of S-CKD602 is presented in Fig. 2A and B. The sum total pharmacokinetic parameters after administration of S-CKD602 are presented in Table 1. After administration of nonliposomal CKD-602, the plasma concentration-versus-time profile of CKD-602 peaked at 0.083 h (5 min) after administration, had a biphasic elimination profile, and was detectable at 72 h after administration. The concentration-versus-time profiles of sum total CKD-602 in all tissues were similar to the profile in plasma. Consistent with the tissue distribution of liposomal encapsulated drugs, the highest exposures of sum total CKD-602 in tissues after administration of S-CKD602

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### Table 1. Sum total pharmacokinetic parameters after administration of S-CKD602 and nonliposomal CKD-602 in mice bearing A375 tumors

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma</th>
<th>Tumor</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Fat</th>
<th>Muscle</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-CKD602*</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0-\infty} (ng/mL•h)</td>
<td>201,929</td>
<td>13,194</td>
<td>39,667</td>
<td>18,919</td>
<td>42,294</td>
<td>4,321</td>
<td>3,399</td>
<td>2,170</td>
</tr>
<tr>
<td>C_{max} (ng/mL)</td>
<td>18,246</td>
<td>280</td>
<td>2,090</td>
<td>707</td>
<td>1,657</td>
<td>281</td>
<td>126</td>
<td>205</td>
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<tr>
<td>T_{max} (h)</td>
<td>0.08</td>
<td>7.0</td>
<td>4</td>
<td>0.5</td>
<td>0.5</td>
<td>0.08</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>T_{last} (h)</td>
<td>72</td>
<td>72</td>
<td>48</td>
<td>72</td>
<td>72</td>
<td>72</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Nonliposomal CKD-602 †</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0-\infty} (ng/mL•h)</td>
<td>9,117</td>
<td>11,661</td>
<td>68,620</td>
<td>56,267</td>
<td>23,124</td>
<td>8,280</td>
<td>24,485</td>
<td>859</td>
</tr>
<tr>
<td>C_{max} (ng/mL)</td>
<td>7,344</td>
<td>3,688</td>
<td>69,000</td>
<td>68,497</td>
<td>14,724</td>
<td>8,807</td>
<td>12,383</td>
<td>572</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>0.08</td>
<td>0.50</td>
<td>0.083</td>
<td>0.08</td>
<td>0.25</td>
<td>0.083</td>
<td>0.08</td>
<td>0.08</td>
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<tr>
<td>T_{last} (h)</td>
<td>7</td>
<td>24</td>
<td>16</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>7</td>
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</tbody>
</table>

* S-CKD602 was administered at 1 mg/kg i.v. push × 1 via tail vein.
† AUC from 0 to last time point.
‡ Nonliposomal CKD-602 was administered at 30 mg/kg i.v. push × 1 via tail vein.
were in the spleen, tumor, and liver. The distribution of S-CKD602 from 0 to 24 h was 1.6-fold greater in fat compared with muscle. In addition, the overall distribution of S-CKD602 was 1.3-fold greater in fat compared with muscle. The ratio of CKD-602 sum total exposure in fat to muscle was 3.8-fold higher after administration of S-CKD602 compared with nonliposomal CKD-602.

**Plasma and tumor disposition of encapsulated and released CKD-602.** We compared the plasma, tumor, and tumor ECF pharmacokinetic disposition of CKD-602 after administration of nonliposomal CKD-602 and S-CKD602 (Table 2). The plasma, tumor, and tumor ECF disposition of CKD-602 after administration of nonliposomal CKD-602 is presented in Fig. 3. The concentrations of sum total CKD-602 were higher in plasma compared with tumor from 0.083 h (5 min) to 2 h and then were higher in tumor compared with plasma from 7 to 24 h. The concentration-versus-time profile of CKD-602 in tumor ECF was detectable from 10 min to 19.25 h and was consistent with the profile of sum total CKD-602 in tumor homogenates. In addition, the concentration of CKD-602 in tumor ECF varied 4- to 5-fold at individual time points during the collection intervals of 0 to 2 h, 4 to 7 h, and 16 to 20 h. The absolute difference in the CKD-602 measured in samples obtained from tumor homogenate (11,661 ng/mL•h) and tumor ECF (639 ng/mL•h) may be due to binding of CKD-602 to plasma proteins or proteins within the tumor matrix.

The plasma, tumor, and tumor ECF disposition of CKD-602 after administration of S-CKD602 is presented in Fig. 4. The concentrations of sum total and encapsulated CKD-602 were detectable from 5 min to 72 h and the released CKD-602 was detectable from 5 min to 48 h after administration of S-CKD602. The concentration-versus-time profile of released CKD-602 was similar to the profiles of sum total and encapsulated CKD-602 from 0 to 75 h. In addition, the ratio of released CKD-602 to sum total or encapsulated CKD-602 was relatively consistent over the same time period. These results suggest that the release of CKD-602 from the liposome is constant. Approximately 82% of CKD-602 remains encapsulated in plasma, as estimated by the ratio of released CKD-602 AUC to sum total CKD-602 AUC or the difference between sum total CKD-602 AUC and encapsulated CKD-602 AUC.

After administration of S-CKD602, the concentration-versus-time profile of sum total CKD-602 measured in tumor homogenates peaked at 7 h and remained relatively constant from 7 to 48 h. The concentration-versus-time profile of CKD-602 in tumor ECF was detectable from 10 min to 75.25 h after administration of S-CKD602, which is significantly greater than after administration of nonliposomal CKD-602. The concentration-versus-time profile of CKD-602 in tumor ECF was consistent with the profile of sum total CKD-602 in tumor homogenates. In addition, the concentration of CKD-602 in tumor ECF varied ~10-fold at individual time points during each of the collection intervals. The difference in the CKD-602 measured in samples obtained from

**Table 2. Plasma, tumor, and tumor ECF pharmacokinetic parameters for S-CKD602 and nonliposomal CKD-602 in mice bearing A375 human melanoma xenografts**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>S-CKD602*</th>
<th>Nonliposomal CKD-602</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum total AUC&lt;sub&gt;0-∞&lt;/sub&gt; (ng/mL•h)</td>
<td>201,929</td>
<td>9,117</td>
</tr>
<tr>
<td>Encapsulated AUC&lt;sub&gt;0-∞&lt;/sub&gt; (ng/mL•h)</td>
<td>165,717</td>
<td>—</td>
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<tr>
<td>Released AUC&lt;sub&gt;0-∞&lt;/sub&gt; (ng/mL•h)</td>
<td>36,905</td>
<td>—</td>
</tr>
<tr>
<td><strong>Tumor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum total AUC&lt;sub&gt;0-∞&lt;/sub&gt; (ng/mL•h)</td>
<td>13,194</td>
<td>11,661</td>
</tr>
<tr>
<td>ECF AUC&lt;sub&gt;0-∞&lt;/sub&gt; (ng/mL•h)</td>
<td>187&lt;sup&gt;b&lt;/sup&gt;</td>
<td>639</td>
</tr>
<tr>
<td>Time &gt;1 ng/mL in tumor ECF (h)</td>
<td>&gt;72</td>
<td>~20</td>
</tr>
</tbody>
</table>

*S-CKD602 was administered at 1 mg/kg i.v. push × 1 via tail vein.
Nonliposomal CKD-602 was administered at 30 mg/kg i.v. push × 1 via tail vein.
AUC from 0 to last time point.

![Fig. 2.](image-url) A and B, concentration-versus-time profiles of sum total CKD-602 in plasma, tumor, and tissues after administration of S-CKD602. The plasma, tumor, and tissue profiles from 0 to 72 h and 0 to 16 h are presented in A and B, respectively. Samples were obtained after administration of vehicle and at 5 min, 0.25, 0.5, 1, 2, 4, 7, 16, 24, 48, and 72 h after administration. Points, mean of three mice. The CV% for the concentrations at each time point for all samples was <25%.
tumor homogenate (13,194 ng/mL h) and tumor ECF (187 ng/mL h) may be due to several factors. Those factors include slow release of CKD-602 from the liposome and binding of CKD-602 to plasma proteins or proteins within the tumor matrix because the tumor ECF samples were obtained using microdialysis methods, which can only recover the released non–albumin bound drug.

Discussion

We have previously evaluated the disposition of released drug in plasma and tumor ECF after administration of a liposomal anticancer agent; however, this is the first study reporting prolonged exposure of released drug in tumor ECF after administration of a STEALTH liposomal anticancer agent (18). In addition, this is the first study evaluating the distribution of a STEALTH liposomal agent compared with a nonliposomal drug to fat and muscle. The results of this study strongly suggest that S-CKD602 provides pharmacokinetic advantages in plasma and tumors when compared with the nonliposomal formulation of CKD-602 at 1/30th the dose. In addition, the results of our study are consistent with the improved antitumor efficacy and therapeutic index of S-CKD602 compared with nonliposomal CKD-602 (11).

The ideal pharmacologic characteristics of a liposomal, nanoparticle, or conjugated anticancer agent are prolonged circulation of the encapsulated drug in the blood or plasma, high tumor delivery, and the release of drug from the carrier into the tumor ECF (6, 16, 17). S-CKD602 meets all of these pharmacologic criteria. The sum total plasma exposure of S-CKD602 was ~25-fold greater than nonliposomal CKD-602. After administration of S-CKD602, 82% of the CKD-602 remained encapsulated in plasma. The overall tumor delivery, as measured by the exposure of sum total CKD-602 measured in tumor homogenates, was similar after administration of S-CKD602 and nonliposomal CKD-602; however, the duration of exposure was ~3-fold longer for S-CKD602 compared with nonliposomal CKD-602. Moreover, the time the concentrations of CKD-602 were >1 ng/mL in the tumor ECF was 3.6-fold longer after S-CKD602 compared with nonliposomal CKD-602 (12–15). The importance of detecting released drug in the tumor ECF after administration of a liposomal anticancer agent is that the encapsulated drug cannot penetrate into the cell and thus it is an inactive prodrug, and that only the released drug can penetrate into the cell and thus is active (6, 18). The importance of the duration of time the concentrations exceeds 1 ng/mL is based on studies evaluating the threshold concentration associated with in vitro cytotoxicity for other camptothecins analogues (2, 12). These results are consistent with the antitumor response to camptothecin analogues, which is related to the duration of exposure to cytotoxic concentrations (2, 12).

After administration of nonliposomal CKD-602, the highest exposure was in the liver and kidney, which is consistent with the clearance of camptothecin analogues (21, 22). After administration of S-CKD602, the highest exposures were in the spleen and liver. The high exposure of drug in spleen after administration of S-CKD602 is consistent with previous studies of conventional and pegylated liposomal drugs and is believed to be due to the presence of the reticuloendothelial system in these tissues (6, 18). Thus, the mPEG coating on STEALTH liposomes does not prevent the clearance via the reticuloendothelial system but slows the clearance of the pegylated drugs via the reticuloendothelial system compared with conventional or nonpegylated liposomes (6, 18). The only way to fully evaluate the extent by which the reticuloendothelial system clears pegylated versus nonpegylated liposomes is to evaluate the plasma, spleen, and liver disposition of each type of liposomal formulation; however, nonpegylated liposomal formulation of CKD-602 has not been developed. Because the activity of the reticuloendothelial system may be a factor that affects
delivery and release of drug from liposomes in plasma and tissue, it may also affect the delivery of liposomal drugs to tumors (6). However, these factors are currently unclear and further exploration of the reticuloendothelial system function and activity as related to the disposition of liposomal anti-cancer agents in tissues and tumors needs to be evaluated (6).

Previous studies evaluating the pharmacokinetics and tissue distribution of a liposomal encapsulated drug have not evaluated the disposition of drug in fat and muscle. The distribution of nonliposomal CKD-602 was greater in muscle compared with fat, whereas the distribution of CKD-602 was greater in fat compared with muscle. Because ~18% of the CKD-602 is released into plasma after administration of S-CKD602, some of the exposure in muscle after administration of S-CKD602 may be associated with the released drug relative to the encapsulated CKD-602. Thus, the results of our study may be an underestimation of the difference in the distribution of CKD-602 to fat and muscle after administration of S-CKD602. These results suggest that the body composition and catabolism of S-CKD602 in patients may affect the disposition of S-CKD602 and released CKD-602.

The brain exposure of CKD-602 was 2.4-fold higher after administration of S-CKD602 compared with nonliposomal CKD-602. This increased penetration of drug after administration of liposomal drugs has also been reported for liposomal formulations of doxorubicin (23, 24). The mechanism by which liposomal agents penetrate into the brain is unknown. The greater exposure of CKD-602 in the brain after S-CKD602 compared with nonliposomal CKD-602 and the activity of camptothecin analogues in patients with brain tumors suggest that S-CKD602 should be evaluated in this patient population.

The development of STEALTH liposomes was based on the discovery that incorporation of PEG-lipids into liposomes yields preparations with prolonged plasma exposure, superior tumor delivery, and increased antitumor effect compared with the nonliposomal formulation of the drug (6, 18). CKD-602 has all of these pharmacologic and cytotoxic advantages. In addition, these advantages are associated with administration of a single i.v. dose of S-CKD602, whereas, as with other camptothecin analogues, the nonliposomal formulation of CKD-602 needs to be administered for several consecutive days to achieve antitumor activity (15, 20–22). Thus, based on the preclinical studies presented here, S-CKD602 may also have logistic advantage over topotecan, which is administered i.v. daily for 5 days repeated every 21 days in the treatment of ovarian cancer and small-cell lung cancer (21, 22).

References

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Plasma, Tumor, and Tissue Disposition of STEALTH Liposomal CKD-602 (S-CKD602) and Nonliposomal CKD-602 in Mice Bearing A375 Human Melanoma Xenografts


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