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# Activity of SQ641, a Capuramycin Analog, in a Murine Model of Tuberculosis[▽]

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**New delivery vehicles and routes of delivery were developed for the capuramycin analogue SQ641. While this compound has remarkable in vitro potency against *Mycobacterium tuberculosis*, it has low solubility in water and poor intracellular activity. We demonstrate here that SQ641 dissolved in the water-soluble vitamin E analogue α-tocopheryl polyethylene glycol 1000 succinate (TPGS) or incorporated into TPGS-micelles has significant activity in a mouse model of tuberculosis.**

SQ641 is an analogue of capuramycin (CM), a naturally occurring nucleoside-based compound produced by *Streptomyces griseus*. It was derived from a library of over 7,000 CM analogues created by Daiichi-Sankyo (Japan) and was identified as the most potent of the translocase I (TL1) inhibitors, with good activity against mycobacteria (2–4, 8, 9). The target of CM and its analogues is TL1, an essential enzyme for biosynthesis of bacterial cell walls. It is an ideal drug target because inhibition of TL1 leads to cell death and the enzyme is unique to bacteria, reducing the likelihood of toxicity.

Sequella licensed the rights to develop the CM analogue library and extended the studies of SQ641. Following on the work performed by Daiichi-Sankyo, we demonstrated (7, 8) that SQ641 shows good activity against both *Mycobacterium tuberculosis* (MIC = 1.0 μg/ml) and *Mycobacterium avium* complex (MIC = 0.016 to 16 μg/ml) bacteria and kills *M. tuberculosis* faster than many other antituberculosis (anti-TB) drugs, including isoniazid (INH) and rifampin (rifampicin). The SQ641 compound has an extraordinary postantibiotic effect of 55 h against *M. tuberculosis* and is active against multidrug-resistant *M. tuberculosis*. SQ641 is strongly synergistic with ethambutol, streptomycin, and SQ109, Sequella's lead antitubercular drug in clinical trials, and is effective in preventing the development of drug-resistant mutants of *M. tuberculosis*.

Unfortunately, SQ641 has limited water solubility and is rapidly expelled from infected cells via P glycoprotein-mediated rapid efflux (4). These limitations result in only modest intracellular activity against *M. tuberculosis* and poor in vivo activity. In the rapid mouse TB model (6), SQ641 in aqueous solution did not completely prevent body weight loss, and in the chronic model of TB, SQ641 was only able to reduce CFU in the lungs by 0.7 log, compared to the levels for the infected and untreated control groups (data not shown).

We were able to overcome these shortcomings by the addition of α-tocopheryl polyethylene glycol 1,000 succinate (TPGS), a water-soluble vitamin E analogue (Eastman, Kingsport, TN). We previously published data demonstrating that formulations of TPGS-solubilized SQ641 and TPGS-based SQ641 micelles improved SQ641 solubility and demonstrated higher intracellular activity than SQ641 in aqueous solution (7). In this study, we investigated the activity of these TPGS-based formulations of SQ641 in the chronic mouse model of TB.

C57BL/6 female mice were inoculated intravenously (i.v.) with *M. tuberculosis* H37Rv. Three weeks following infection, treatment was initiated with either a TPGS solution of SQ641 or SQ641-containing micelles, prepared as previously described (7). Drugs were administered intraperitoneally (i.p.) (five times per week) or i.v. (two times per week) for a total of 4 weeks. At the end of treatment, one group of mice was sacrificed for every tested drug and concentration, and 10-fold dilutions of lung homogenates were plated on 7H10 agar to determine the number of CFU of *M. tuberculosis* (1, 5). *M. tuberculosis*-infected untreated and INH-treated mice were used as controls.

Table 1 shows the results from treatment with TPGS-solubilized SQ641. Three weeks following infection with $5 \times 10^4$ CFU *M. tuberculosis*, mice were treated for 4 weeks with SQ641-TPGS at a dose of 50 mg/kg of body weight given i.p. (five times per week) or SQ641-TPGS at a dose of 25 mg/kg administered i.v. (two times per week). Untreated mice were used as controls, and mice treated with INH at 25 mg/kg given per os (five times per week) served as positive controls. For all doses, TPGS was used as a 1.75% water solution. Lung CFU data (Table 1) suggested that SQ641-TPGS had remarkable activity when administered i.p., reducing $\log_{10}$ numbers of CFU in lungs from 8.09 (control) to 6.70 at 50 mg/kg. SQ641-TPGS administered i.v. showed even better activity, reducing the $\log_{10}$ numbers of CFU in lungs from 8.09 to 6.10 at 25 mg/kg.

In the same in vivo study, we also evaluated the efficacy of TPGS-SQ641 micelles delivered i.p. (five times per week) or i.v. (two times per week) (Table 1). SQ641-micelles delivered i.p. reduced $\log_{10}$ numbers of CFU in lungs from 8.09 to 6.15 (100 mg/kg). When administered i.v., only 8 doses of TPGS-SQ641 administered to mice two times per week for a total of

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TABLE 1. Activity of SQ641 in 1.75% TPGS solution and in Micelles in a C57BL/6 mouse model of TB[a]

| Treatment group | Formulation | Route (frequency) of drug administration | Log no. of CFU/lung (mean ± SD) | *P* for statistical difference[b] from: | | |
|---|---|---|---|---|---|---|
| | | | | EC | LC | INH |
| Untreated | | | | | | |
| Early control | NO | | 7.46 ± 0.21 | | | |
| Late control | NO | | 8.09 ± 0.18 | | | |
| Treated with INH | | | | | | |
| 25 mg/kg | Solution | Gavage (5/wk) | 5.10 ± 0.59 | | | |
| Treated with SQ641 | | | | | | |
| 50 mg/kg | Solution | i.p. (5/wk) | 6.70 ± 0.20 | 0.03 | <0.0015 | |
| 25 mg/kg | Solution | i.v. (2/wk) | 6.10 ± 0.34 | | <0.0015 | 0.075 |
| 100 mg/kg | Micelles | i.p. (5/wk) | 6.15 ± 0.35 | | <0.0015 | |
| 50 mg/kg | Micelles | i.v. (2/wk) | 6.01 ± 0.21 | | <0.0015 | 0.015 |

[a] Therapy with drugs was initiated 3 weeks after *M. tuberculosis* H37Rv infection. Mice were treated with drugs for 4 weeks. NO, no treatment; EC, early control; LC, late control.

[b] Statistical significance was calculated by ANOVA and Student's *t* test; for multiple comparisons, Bonferroni correction was used. Differences were considered significant if *P* was <0.05.

4 weeks lowered CFU counts in lungs by 2.08 log (50 mg/kg). INH administered at 25 mg/kg reduced $\log_{10}$ numbers of CFU in lungs to 5.10. Two i.v. injections of SQ641-micelles per week at a dose of 50 mg/kg resulted in the CFU reduction closest to that for INH treatment.

We also tested combination treatments of SQ641 with INH. These combinations given i.v. were more effective than each drug separately. SQ641-TPGS solution at 25 mg/kg with INH (25 mg) reduced log numbers of CFU in lungs to 4.64, and SQ641-micelles at 25 mg/kg reduced the level to 4.46 (not shown), compared to INH alone ($\log_{10}$ CFU = 5.10). However, statistical significance as determined with the Bonferroni posttest showed *P* values of >0.05.

In other experiments (not shown), we determined that SQ641 in 2.5% TPGS solution as well as TPGS-micelles containing SQ641 at high doses reduced lung CFU to levels comparable to that of INH (not shown). In addition, SQ641 had better efficacy at higher TPGS concentrations (not shown), suggesting an important role for TPGS in overcoming P glycoprotein-mediated drug efflux. However, these higher concentrations of TPGS (greater than 2.5%) can cause pathological alterations in the abdominal cavity of mice and therefore are not practical as a delivery vehicle for SQ641.

The results of this study suggest that the hurdles to an in vivo realization of the remarkable in vitro potency of SQ641 may be overcome. Utilization of appropriate solvents for assistance with solubilization (TPGS and/or others) and carriers such as micelles, liposomes, and nanoparticles has the potential to make this compound a very effective anti-TB drug.

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